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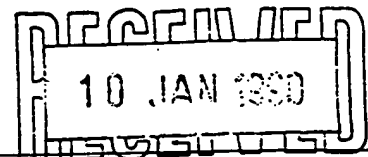
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The filing date provisionally given to the application is

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Applicants:

Celltech Limited



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HUMANISED ANTIBODIES

The present invention relates to humanised antibody molecules (HAMS), to processes for their production using recombinant DNA technology, and to their therapeutic uses.

In the present application, the term "recombinant antibody molecule" (RAM) is used to describe an antibody produced by a process involving the use of recombinant DNA technology, including any analogues of natural immunoglobulins or their fragments. The term "humanised antibody molecule" (HAM) is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise either complete variable domains fused onto constant domains or complementarity determining regions grafted onto appropriate framework regions in the variable domains. The abbreviation "MAB" is used to indicate a monoclonal antibody.

In the description, reference is made to a number of publications by number. The publications are listed in numerical order at the end of the description.

Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, (Fab')₂ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of techniques for the preparation of monoclonal antibodies of defined specificity (1). However, most MAbs are produced by fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. Thus, in practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

Some early methods for carrying out such a procedure are described in EP-A-0 171 496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), EP-A-0 194 276 (Celltech Limited) and WO-A-8 702 671 (Int. Gen. Eng. Inc.). The Celltech application discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. It also shows the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin, and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin.

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. There are 3 CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable regions. The present invention relates to HAMs prepared according to this alternative approach, i.e. CDR-grafted HAMs.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells respectively were humanised by CDR-grafting are shown by Verhoeven et al (2) and Riechmann et al (3).

In the latter case (Riechmann et al) it was found that transfer of the CDR regions alone (as defined by Kabat refs. 4 and 5) was not sufficient to provide

satisfactory antigen binding activity in the CDR-grafted product. Riechmann et al found that it was necessary to convert a serine residue at position 27 of the human sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having satisfactory antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicate that changes to residues of the human sequence outside the CDR regions, in particular in the loop adjacent to CDR1, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens.

In recent years a number of rodent MABs have been developed for therapeutic applications. For instance, OKT3 a mouse IgG2a/k MAB which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in the USA as an immunosuppressant in the treatment of acute allograft rejection (Chatenond et al (1986) J. Immunol., 137, 830-838, and Jeffers et al (1986) Transplantation, 41, 572-578). However, in view of the rodent nature of this and other such MABs, a significant HAMA response which may include a major anti-idiotypic component, builds up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response by suitable humanisation or other recombinant DNA manipulation of these very useful antibody and thus enlarge their areas of use.

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We have further investigated the preparation of CDR-grafted HAMS and have identified residues within the framework of the variable region (i.e. outside both the Kabat CDRs and structural loops of the variable regions) the amino acid identities of which are important for obtaining CDR-grafted products with satisfactory binding affinity.

Accordingly, in a first aspect the invention provides a CDR-grafted antibody heavy chain having a variable region domain comprising human framework and non-human (rodent) antigen binding regions wherein the human framework comprises non-human (rodent) residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

Preferably, the antigen binding regions of the CDR-grafted heavy chain variable domain comprise CDRs corresponding to the Kabat CDR at CDR2 (residues 50-65), the structural loop residues at CDR3 (residues 95-100) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35).

The invention also provides in a second aspect a CDR-grafted antibody light chain having a variable region domain comprising human framework and non-human (rodent) antigen binding regions wherein the human framework comprises non-human (rodent) residues at at least one of positions 1 and/or 3 and 46 and/or 47.

Preferably, the antigen binding regions of the CDR-grafted light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34) and CDR2 (residues 50-56) and the structural loop residues at CDR3 (residues 91-96).

The invention further provides a CDR-grafted HAM comprising at least one CDR-grafted heavy chain and at least one CDR-grafted light chain according to the first and second aspects of the invention.

The residue designations given above and elsewhere in the present specification are numbered according to the Kabat numbering (refs. 4 and 5).

Preferably the CDR-grafted heavy chain comprises non-human (rodent) residues at positions 23 and/or 24, 48 and/or 49 and 71 and/or 73. Preferably, the CDR-grafted light chain comprises non-human (rodent) residues at positions 46 and/or 47.

Preferably the CDR-grafted antibody heavy and light chains and HAM are produced by recombinant DNA technology.

The HAM of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as the Fab or (Fab')₂ fragment; a light chain or heavy chain monomer or dimer; or any other molecule with the same specificity as the original non-human (rodent) antibody. Similarly the CDR-grafted heavy and light chain variable region may be combined with other antibody domains as appropriate.

Alternatively, the heavy or light chains or HAM of the present invention may have attached to them an effector or reporter molecule. For instance, they may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used

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to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by an enzyme or toxin molecule.

For CDR-grafted products of the invention, appropriate variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of human framework used is of the same/similar class/type as the donor antibody. Advantageously, the framework is chosen to maximise/optimize homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. It will be appreciated that in some cases that the non-human and human amino acid residues, identified above in connection with the first and second aspects of the invention, may be the same and thus no change of the human framework to the corresponding non-human framework residue is required.

Also human constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domain. In particular, IgG human constant region domains may be used especially of the IgG1 and IgG3 isotypes, when the HAM is intended for therapeutic uses.

However, the remainder of the HAM need not comprise only protein sequences from the human immunoglobulin. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence

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encoding the amino acid sequence of a polypeptide effector or reporter molecule.

Thus, according to a further aspect the present invention provides a process for producing an anti-CD3 HAM which process comprises:

- (a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy or light chain according to the first or second aspect of the invention;
- (b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light or heavy chain according to the second or first aspect of the invention;
- (c) transfecting a host cell with the or each vector; and
- (d) culturing the transfected cell line to produce the HAM.

The cell line may be transfected with two vectors, the first vector containing an operon encoding a light chain-derived polypeptide and the second vector containing an operon encoding a heavy chain-derived polypeptide. Preferably, the vectors are identical except in so far as the coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed.

Alternatively, a single vector may be used, the vector including the sequences encoding both light chain- and heavy chain-derived polypeptides.

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The DNA in the coding sequences for the light and heavy chains may comprise cDNA or genomic DNA or both.

The present invention also includes cloning and expression vectors and transfected cell lines used in the process of the invention, therapeutic and diagnostic compositions comprising the variable domains or the HAM of the invention and uses of such compositions in therapy and diagnosis.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se and form no part of the invention. Such methods are shown, for instance, in references 6 and 7.

The present invention is now described, by way of example only, with reference to the accompanying Figures 1 - 29.

MATERIAL AND METHODS

1. INCOMING CELLS

Hybridoma cells producing antibody OKT3 were provided by Ortho (seedlot 4882-1) and were grown up in antibiotic free Dulbecco's Modified Eagles Medium (DMEM) supplemented with glutamine and 5% foetal calf serum, and divided to provide both an overgrown supernatant for evaluation and cells for extraction of RNA. The overgrown supernatant was shown to contain 250 ug/mL murine IgG2a/kappa antibody. The supernatant was negative for murine lambda light chain and IgG1, IgG2b, IgG3, IgA and IgM heavy chain. 20mL. of supernatant was sent to Ortho to confirm that the antibody present was OKT3.

2. MOLECULAR BIOLOGY PROCEDURES

Basic molecular biology procedures were as Maniatis et al.(ref. 6) with, in some cases minor modifications. DNA sequencing was performed as described in Sanger et al.(ref. 7) and the Amersham International Plc sequencing handbook. Site directed mutagenesis was as described in Kramer et al.(ref.8) and the Anglian Biotechnology Ltd handbook. COS cell expression and metabolic labelling studies were as described in Whittle et al. (ref. 9)

3. RESEARCH ASSAYS

3.1 ASSEMBLY ASSAYS

Assembly assays were performed on supernatants from transfected COS cells to determine the amount of intact IgG present.

3.1.1 COS CELLS TRANSFECTED WITH MOUSE OKT3 GENES

The assembly assay for intact mouse IgG in COS cell supernatants was an ELISA with the following format:-

96 well microtitre plates were coated with F(ab')₂ goat anti mouse IgG Fc. The plates were washed in water and samples added for 1 hour at room temperature. The plates were washed and F(ab')₂ goat anti mouse IgG F(ab')₂ (HRPO conjugated) was then added. Substrate was added to reveal the reaction.

UPC10, a mouse IgG2a myeloma, was used as a standard.

3.1.2 • COS CELLS TRANSFECTED WITH CHIMAERIC OR CDR GRAFTED OKT3 GENES

The assembly assay for intact humanised OKT3 in COS cell supernatants was an ELISA with the following format:-

96 well microtitre plates were coated with F(ab')₂ goat anti-human IgG Fc. The plates were washed and samples added for 1 hour at room temperature. The plates were washed and monoclonal mouse anti-human kappa chain was added for 1 hour at room temperature.

The plates were washed and F(ab')₂ goat anti mouse IgG Fc (HRPO conjugated) was added. Substrate was added to reveal the reaction.

Chimaeric B72.3 (IgG4) was used as a standard. The use of a monoclonal anti-kappa chain in this assay allows grafted antibodies to be read from the chimaeric standard.

3.2 ASSAY FOR OKT3 ANTIGEN BINDING ACTIVITY

Material from COS cell supernatants was assayed for OKT3 antigen binding activity onto CD3 positive cells in a direct assay. The procedure was as follows:-

HUT 78 cells (human T cell line, CD3 positive) were maintained in culture. Monolayers of HUT 78 cells were prepared onto 96 well ELISA plates using poly-L-lysine and glutaraldehyde. Samples were added to the monolayers for 1 hour at room temperature.

The plates were washed gently using PBS. F(ab')₂ goat anti-human IgG Fc (HRPO conjugated) or F(ab')₂ goat anti mouse IgG Fc (HRPO conjugated) was added as appropriate for humanised or mouse samples. Substrate was added to reveal the reaction.

The negative control for the cell-based assay was chimaeric B72.3. The positive control was mouse Orthomune OKT3 or chimaeric OKT3, when available. This cell-based assay was difficult to perform and gave poorly reproducible results with a high background.

4. cDNA LIBRARY CONSTRUCTION

4.1 mRNA PREPARATION AND cDNA SYNTHESIS

OKT3 producing

Cells were grown as described above and 1.2×10^9 cells

harvested and mRNA extracted using the guanidinium/LiCl extraction procedure. cDNA was prepared by priming from Oligo-dT to generate full length cDNA. The cDNA was methylated and EcoR1 linkers added for cloning.

4.2 LIBRARY CONSTRUCTION

The cDNA library was ligated to pSP65 vector DNA which had been EcoR1 cut and the 5' phosphate groups removed by calf intestinal phosphatase (EcoRI/CIP). The ligation was used to transform high transformation efficiency *Escherichia coli* (*E.coli*) HB101. A cDNA library was prepared. 3600 colonies were screened for the light chain and 10000 colonies were screened for the heavy chain.

5. SCREENING

E.coli colonies positive for either heavy or light chain probes were identified by oligonucleotide screening using the oligonucleotides :

5' TCCAGATGTAACTGCTCAC for the light chain, which is complementary to a sequence in the mouse kappa constant region, and 5' CAGGGGCCAGTGGATGGATAGAC for the heavy chain which is complementary to a sequence in the mouse IgG2a constant CH1 domain region. 12 light chain and 9 heavy chain clones were identified and taken for second round screening. Positive clones from the second round of screening were grown up and DNA prepared. The sizes of the gene inserts were estimated by gel electrophoresis and inserts of a size capable of containing a full length cDNA were subcloned into M13 for DNA sequencing.

6. DNA SEQUENCING

Clones representing four size classes for both heavy and light chains were obtained in M13. DNA sequence for the 5' untranslated regions, signal sequences, variable regions and 3' untranslated regions of full length cDNA s were obtained (Figs 1 and 2).

ANALYSIS OF SEQUENCES

DNA sequences from cDNA's were compared with RNA sequences

provided by Ortho. The cDNA sequences included 5' untranslated region sequence as well as signal peptide sequence. The 3' untranslated region was also sequenced. A single coding difference was observed at position 9 in the heavy chain where the mRNA suggested a Proline but the cDNA sequence read as an Alanine. The cDNA sequence was used for further analysis.

The light chain is a member of the mouse V_L subgroup VI and uses a J_{K4} minigene. The heavy chain is probably a member of the mouse V_H subgroup II, most probably IIb, although it also has significant homology to the consensus for group Va which itself is very homologous to subgroup II. The D region is currently unclassified and the JH region is J_{H2} (Figs 3 and 4).

The light chain shows a high degree of homology to the Ox-1 germline gene and to the published antibodies 45.21.1, 14.6b.1 and 26.4.1. The heavy chain shows reasonable homology to a subgroup of the J558 family including 14.6b.1. These combinations of light and heavy chain genes have previously resulted in antibodies with affinity for alpha-1-6 dextran (Sikder et al. (ref.10) Wallick et al. (ref.11)).

The heavy chain has the sequence Asparagine (Asn)- Proline (Pro)- Serine (Ser) in CDR2. Normally Asn-X-Ser would be a potential glycosylation site, but when X is Pro these sites tend not to be glycosylated.

8. CONSTRUCTION OF cDNA EXPRESSION VECTORS

Celltech expression vectors are based on the plasmid pEE6hCMV (Fig. 5) (ref. 12). A polylinker for the insertion of genes to be expressed has been introduced after the major immediate early promoter/enhancer of the human Cytomegalovirus (hCMV). Marker genes for selection of the plasmid in transfected eukaryotic cells can be inserted as BamHI cassettes in the unique BamHI site of pEE6 hCMV. It is usual practice to insert the *neo* and *gpt* markers prior to insertion of the gene of interest, whereas the GS marker is inserted last because of the presence of internal EcoRI sites in the cassette.

The selectable markers are expressed from the SV40 late promoter which also provides an origin of replication so that the vectors can be used for expression in the COS cell transient expression system.

The mouse sequences were excised as EcoRI fragments and cloned into either EE6-hCMV-*neo* for the heavy chain (Fig 6) and into EE6-hCMV-*gpt*

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for the light chain (Fig 7).

9. EXPRESSION OF cDNA'S IN COS CELLS

Plasmids pJA135 (Fig 7) and pJA136 (fig 6) were co-transfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to T-cell enriched peripheral blood lymphocytes. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains.

10. CONSTRUCTION OF CHIMAERIC GENES

Construction of chimaeric genes followed a previously described strategy (Whittle et al (ref. 9)). A restriction site near the 3' end of the variable domain sequence is identified and used to attach an oligonucleotide adapter coding for the remainder of the mouse variable region and a suitable restriction site for attachment to the constant region of choice.

10.1 LIGHT CHAIN GENE CONSTRUCTION- VERSION 1

The mouse light chain cDNA sequence showed an *Ava*I site near the 3' end of the variable region (Fig 8). The majority of the sequence of the variable region was isolated as a 376 bp. *Eco*RI-*Ava*I fragment. An oligonucleotide adapter was designed to replace the remainder of the 3' region of the variable region from the *Ava*I site and to include the 5' residues of the human constant region up to and including a unique *Nar*I site which had been previously engineered into the constant region.

TOP STRAND 5' TCGGGGACAAAGCTTGAATAAACAGAACTGTGGCGG 3'

BOTTOM STRAND 3' CCTGTTTCGAACTTTATTTGTCTTGACACCGCCGC 5'

A *Hind*III site, shown in bold type within the oligonucleotide sequence above, was introduced to act as a marker for insertion of the linker.

The linker was ligated to the V_L fragment and the 413 bp *Eco*RI-*Nar*I adapted fragment was purified from the ligation mixture.

The constant region was isolated as an *Nar*I-BamHI fragment from an M13 clone NW361 and was ligated with the variable region DNA into an *Eco*RI/BamHI/CIP pSP65 treated vector in a three way reaction. Clones were isolated after transformation into *E.coli* and the linker and junction sequences were confirmed by the presence of the *Hind*III site and by DNA sequencing (Fig 9).

10.2 LIGHT CHAIN GENE CONSTRUCTION- VERSION 2

The construction of the first chimaeric light chain gene produces a fusion of mouse and human amino acid sequences at the variable -constant region junction. In the case of the OKT3 light chain the amino acids at the chimaera junction are:

.....Leu-Glu-Ile-Asn-Arg/ -/Thr-Val-Ala -Ala

VARIABLE CONSTANT

This arrangement of sequence introduces a potential site for Asparagine (Asn) linked (N-linked) glycosylation at the V-C junction. As will be seen later, this sequence can be glycosylated. Therefore, a second version of the chimaeric light chain oligonucleotide adapter was designed in which the threonine (Thr), the first amino acid of the human constant region, was replaced with the equivalent amino acid from the mouse constant region, Alanine (Ala).

TOP STRAND 5' TCGGGGACAAAGTTGGAATAAACAGAGCTGTGGCGG 3'

BOTTOM STRAND 3' CCTGTTTCAACCTTTATTTGTCTCGACACCGCCGC 5'

The internal HindIII site present in the version 1 adapter was not included to differentiate the two chimaeric light chain genes.

The variable region fragment was isolated as a 376 bp. EcoRI-AvaI fragment. The oligonucleotide linker was ligated to NarI cut pNW361 and then the adapted 396bp constant region was isolated after recutting the modified pNW361 with EcoRI. The variable region fragment and the modified constant region fragment were ligated directly into EcoRI/CIP treated pEE6hCMVneo.

Initially all clones examined had the insert in the incorrect orientation. Therefore, the insert was re-isolated and recloned to turn the insert round. Several clones with the insert in the correct orientation were obtained and the adapter sequence of one was confirmed by DNA sequencing (Fig10).

10.3 HEAVY CHAIN GENE CONSTRUCTION

10.3.1 CHOICE OF HEAVY CHAIN GENE ISOTYPE

The constant region isotype chosen for the heavy chain was human IgG4.

10.3.2 GENE CONSTRUCTION

The heavy chain cDNA sequence showed a BstI site near the 3' end of the variable region (Fig 11). The majority of the sequence of the variable region was isolated as a 426bp. EcoRI/CIP/BstI fragment. An oligonucleotide adapter was designed to replace the remainder of the

3' region of the variable region from the BanI site up to and including a unique HindIII site which had been previously engineered into the first two amino acids of the constant region.

TOP STRAND 5' GCACCACTCTCACCGTGAGCTC3'

BOTTOM STRAND 3' GTGAGAGTGGCACTCGAGTCGA 5'

The linker was ligated to the V_H fragment and the EcoRI-HindIII adapted fragment was purified from the ligation mixture.

The variable region was ligated to the constant region by cutting mJA91 with EcoRI and HindIII removing the intron fragment and replacing it with the V_H (Fig 12). Clones were isolated after transformation into *E.coli* JM101 and the linker and junction sequences were confirmed by DNA sequencing. (NB. The HindIII site is lost on cloning).

11. CONSTRUCTION OF CHIMAERIC EXPRESSION VECTORS

11.1 neo AND gpt VECTORS

The chimaeric light chain (version 1) was removed from pJA143 (Fig 9) as an EcoRI fragment and cloned into EcoRI/CIP treated pEE6hCMVneo expression vector. Clones with the insert in the correct orientation were identified by restriction mapping (Fig 13).

The chimaeric light chain (version 2) was constructed as described above (see Fig 10).

The chimaeric heavy chain gene was isolated as a 2.5Kbp EcoRI/BamHI fragment and cloned into the EcoRI/BclI/CIP treated vector fragment of pJA97, a derivative of pEE6hCMVgpt (Fig 14).

11.2 GS SEPARATE VECTORS

GS versions of pJA141 (Fig 10) and pJA144 (Fig 14) were constructed by replacing the neo and gpt cassettes by BamHI/SalI/CIP treatment of the plasmids, isolation of the vector fragment and ligation to a GS-containing fragment from the plasmid pRO49 (Figs 15 and 16)

11.3 GS SINGLE VECTOR CONSTRUCTION

Single vector constructions containing the cL, cH and GS genes on one plasmid in the order cL-cH-GS, or cH-cL-GS and with transcription of the genes being head to tail eg. cL>cH>GS were constructed. These plasmids were made by treating pJA179 (Fig 15) or pJA180 (Fig 16) with BamHI/CIP and ligating in a BglII/HindIII hCMV cassette from pJA146 along with either the HindIII/BamHI from pJA141 into pJA180 to give the cH-cL-GS plasmid pJA182 (Fig 17), or the HindIII/BamHI from pJA144 into pJA179 to give the cL-cH-GS plasmid pJA181 (Fig 18).

12. EXPRESSION OF CHIMAERIC GENES

12.1 EXPRESSION IN COS CELLS

The chimaeric antibody plasmids pJA145 (cL) and pJA144 (cH) were cotransfected into COS cells and supernatant from the transient expression experiment was shown to contain assembled antibody which bound to the HUT 78 human T-cell line. Metabolic labelling experiments using ^{35}S methionine showed expression and assembly of heavy and light chains. However the light chain mobility seen on reduced gels (Fig 19) suggested that the potential glycosylation site was being glycosylated. Expression in COS cells in the presence of tunicamycin showed a reduction in size of the light chain to that shown for control chimaeric antibodies and the OKT3 mouse light chain. Therefore JA141 was constructed and expressed. In this case the light chain did not show an aberrant mobility or a size shift in the presence or absence of tunicamycin (Fig 19). This second version of the chimaeric light chain, when expressed in association with chimaeric heavy (cH) chain, produced antibody which showed good binding to HUT 78 cells. In both cases antigen binding was equivalent to that of the mouse antibody.

12.2 EXPRESSION IN CHINESE HAMSTER OVARY (CHO) CELLS

Stable cell lines are being prepared from plasmids pJA141/pJA144 and from pJA179/pJA180, pJA181, and pJA182 by transfection into CHO cells.

13. CDR GRAFTING

The approach taken was to try to introduce sufficient mouse residues into a human variable region framework to generate antigen binding activity comparable to the mouse and chimaeric antibodies.

13.1 VARIABLE REGION ANALYSIS

From an examination of a small database of structures of antibodies and antigen-antibody complexes it is clear that only a small number of antibody residues make direct contact with antigen. Other residues may contribute to antigen binding by positioning the contact residues in favourable configurations and also by inducing a stable packing of the individual variable domains and stable interaction of the light and

heavy chain variable domains.

The residues chosen for transfer can be identified in a number of ways:

- A. By examination of antibody X-ray crystal structures the antigen binding surface can be predominantly located on a series of loops, three per domain, which extend from the B-barrel framework.
- B. By analysis of antibody variable domain sequences, regions of hypervariability (termed the Complementarity Determining Regions (CDRs) by Wu and Kabat (ref. 5)) can be identified. In the most but not all cases these CDRs correspond to, but extend a short way beyond, the loop regions noted above.
- C. Residues not identified by A and B above may contribute to antigen binding directly or indirectly by affecting antigen binding site topology, or by inducing a stable packing of the individual variable domains and stabilising the inter-variable domain interaction. These residues may be identified either by superimposing the sequences for a given antibody on a known structure and looking at key residues for their contribution, or by sequence alignment analysis and noting "idiosyncratic" residues followed by examination of their structural location and likely effects.

13.1.1 LIGHT CHAIN

Figure 20 shows an alignment of sequences for the human framework region REI and the OKT3 light variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1C. REI was chosen as the human framework because the light chain is a Kappa chain and the kappa variable regions show higher homology with the mouse sequences than a lambda light variable region eg KOL (see below). REI was chosen in preference to another kappa light chain because the X-ray structure of the light chain has been determined so that a structural examination of individual residues could be made.

13.1.2 HEAVY CHAIN

Figure 21 shows an alignment of sequences for the human framework region KOL and the OKT3 heavy variable region. The structural loops and CDRs believed to correspond to the antigen binding region are marked. Also marked are a number of other residues which may also contribute to antigen binding as described in 13.1C. KOL was chosen as the heavy chain framework because the X-ray structure has been determined to a better resolution than, for example, NEWM and also

the sequence alignment of OKT3 heavy variable region showed a slightly better homology to KOL than to NEWM.

13.2 DESIGN OF VARIABLE GENES

The variable region domains were designed with mouse variable region optimal codon usage (Grantham and Perrin,(ref.13))and used the B72.3 signal sequences (Whittle et al.(ref.9))The sequences were designed to be attached to the constant region in the same way as for the chimaeric genes described above. Some constructs contained the "Kozak consensus sequence" (Kozak,(ref.14))directly linked to the 5' of the signal sequence in the gene. This sequence motif is believed to have a beneficial role in translation initiation in eukaryotes.

13.3 GENE CONSTRUCTION

To build the variable regions two strategies are available. Either to assemble the sequence using oligonucleotides in a manner similar to Jones et al. (ref. 15) or to simultaneously replace all of the CDRs or loop regions by oligonucleotide directed site specific mutagenesis in a manner similar to Verhoeyen et al. (ref. 2) Both strategies were used and a list of constructions is set out in Table 1. It was noted in several cases that the mutagenesis approach led to deletions and rearrangements in the gene being remodelled, while the success of the assembly approach was very sensitive to the quality of the oligonucleotides. Figs 22a and b and 23a and b show by way of example the nucleotide sequences and procedures required to construct gH341 by site directed mutagenesis and kgH341A by oligonucleotide assembly.

14 CONSTRUCTION OF EXPRESSION VECTORS

Genes were isolated from M13 or SP65 based intermediate vectors and cloned into pEE6hCMVneo for the light chains and pEE6hCMVgpt for the heavy chains in a manner similar to that for the chimaeric genes as described above.

15 EXPRESSION OF CDR GRAFTED GENES

A number of points should be noted.

1. There is no standard for the antigen binding assay when chimaeric or CDR grafted antibody are being measured, except when the heavy chain of the antibody is murine when murine OKT3 can be used as standard with an anti-murine Fc antibody as revealing antibody. Therefore all comparisons of antigen binding assays with chimaeric (c) or CDR grafted (g) genes can only be made within an individual

TABLE 1 CDR GRAFTED GENE CONSTRUCTIONS

CODE	MOUSE SEQUENCE CONTENT	METHOD OF CONSTRUCTION	KOZAK SEQUENCE

LIGHT-CHAIN	ALL HUMAN FRAMEWORK REI		
121	26-32, 50-56, 91-96 inclusive	SDM and gene assembly	+ n.d.
121A	26-32, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	n.d. +
121B	26-32, 50-56, 91-96 inclusive +46, 47	Partial gene assembly	n.d. +
221	24-24, 50-56, 91-96 inclusive	Partial gene assembly	+ +
221A	24-34, 50-56, 91-96 inclusive +1, 3, 46, 47	Partial gene assembly	+ +
221B	24-34, 50-56, 91-96 inclusive +1, 3	Partial gene assembly	+ +
221C	24-34, 50-56, 91-96 inclusive +46, 47	Partial gene assembly	+ +
HEAVY CHAIN	ALL HUMAN FRAMEWORK KOL		
121	26-32, 50-56, 95-100B inclusive	Gene assembly	n.d. +
131	26-32, 50-58, 95-100B inclusive	Gene assembly	n.d. +
141	26-32, 50-65, 95-100B inclusive	Partial gene assembly	+ n.d.
321	26-35, 50-56, 95-100B inclusive	Partial gene assembly	+ n.d.
331	26-35, 50-58, 95-100B inclusive	Partial gene assembly Gene assembly	+ +
341	26-35, 50-65, 95-100B inclusive	SDM Partial gene assembly	+ +
341A	26-35, 50-65, 95-100B inclusive +6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91 (+63=human)	Gene assembly	n.d. +
341B	26-35, 50-65, 95-100B inclusive +48, 49, 71, 73, 76, 78, 88, 91(+63=human)	Gene assembly	n.d. +

KEY

n.d.	not done
SDM	Site directed mutagenesis
Gene assembly	Variable region assembled entirely from oligonucleotides
Partial gene assembly	Variable region assembled by combination of restriction fragments either from other genes originally created by SDM and gene assembly or by oligonucleotide assembly of part of the variable region and reconstruction with restriction fragments from other genes originally created by SDM and gene assembly.

experiment.

2. The cell-based antigen binding assay is not robust and resulting data varies depending on cell binding to the plates and the amount of antibody used. Therefore several experiments are needed to confirm marginal results.

3. The COS cell expression system can give batch to batch variation in antibody yield which has a direct bearing on the results obtained in the antigen binding assay.

Bearing these factors in mind the data can be divided into three groups. Table 2 shows a summary of data for the various constructs.

15.1 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED LIGHT (gL) CHAINS WITH MOUSE HEAVY (mH) OR CHIMAERIC HEAVY (cH) CHAINS.

All gL chains, in association with mH or cH produced reasonable amounts of antibody. Insertion of the Kozak consensus sequence at a position 5' to the ATG (kgL constructs) however, led to a 2-5 fold improvement in net expression (Fig 24a and b). Over an extended series of experiments expression levels were raised from approx 200ng/mL to approx. 500 ng/mL for kgL/cH or kgL/mH combinations.

When direct binding to antigen on HUT 78 cells was measured, a construct designed to include mouse sequence based on loop length (gL121) did not lead to active antibody in association with mH or cH. A construct designed to include mouse sequence based on Kabat CDRs (gL221) demonstrated some weak binding in association with mH or cH (Fig 25B). However when framework residues 1, 3, 46, 47 were changed from the human to the murine OKT3 equivalents based on the arguments outlined in Section 13.1 antigen binding can be demonstrated when both of the new constructs, which were termed 121A and 221A, are coexpressed with cH (Fig 25A and B). When the effects of these residues are examined in more detail it appears that residues 1 and 3 are not major contributing residues as the product of the gL221B gene shows little detectable binding activity in association with cH (Fig 25B). The light chain product of gL221C, in which mouse sequences are present at 46 and 47, shows good binding activity in association with cH (Fig 25 B).

15.2 PRODUCTION OF ANTIBODY CONSISTING OF GRAFTED HEAVY (gH) CHAINS WITH MOUSE LIGHT (mL) OR CHIMAERIC LIGHT(cL) CHAINS.

Expression of the gH genes has proven to be more difficult to achieve than for gL. First, inclusion of the Kozak sequence appears to have

had no marked effect on expression of gH genes (Fig 26). Expression may be slightly improved but not to the same degree as seen for the grafted light chain.

Second, it has proven difficult to demonstrate production of expected quantities of material when the loop choice (amino acid 26-32) for CDR1 is used eg. gH121, 131, 141 (Fig 27) and no conclusions can be drawn about these constructs. Further, in experiments where low antibody production was seen it has not been possible to detect free light chain expression and secretion which would be expected if heavy chain expression was not occurring at all. Therefore the data suggests, but does not confirm, that in these cases the heavy chain is being expressed but the processing of the chain once it has become associated with light chain is aberrant leading to degradation of assembled or partially assembled antibody inside the cell. Experiments to determine gH mRNA levels, or to attempt to demonstrate the presence of antibody within the cells have not been done.

Third, coexpression of the gH341 gene with cL or mL has been variable and has tended to produce lower amounts of antibody than the cH/cL or mH/mL combinations. The alterations to gH341 to produce gH341A and gH341B appear to lead to improved levels of expression (Fig 27 lanes h-k). This may partly be due either to a general increase in the fraction of mouse sequence in the variable region, or to the alteration at position 63 where the residue is returned to the human amino acid Valine (Val) from Phenylalanine (Phe) to avoid possible internal packing problems with the rest of the human framework. This arrangement also occurs in gH331 and gH321.

When gH321 or gH331 are expressed in association with cL, antibody is produced but antibody binding activity has not been detected (Table 2). When the more conservative gH341 gene is used antigen binding can be detected in association with cL or mL, but the activity is only marginally above the background level (Fig 28). When further mouse residues are substituted based on the arguments in 13.1 antigen binding can be clearly demonstrated for the antibody produced when kgH341A and kgH341B are expressed in association with cL (Fig 29).

15.3

PRODUCTION OF FULLY CDR GRAFTED ANTIBODY

The kgL221A gene was co-expressed with kgH341, kgH341A, or kgH341B. For the combination kgH221A/kgH341 very little material was produced in a normal COS cell expression experiment (Fig 29A and C). For the

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combinations kgL221A/kgH341A or kgH221A/kgH341B amounts of antibody similar to gL/cH or cL/cH was produced (Fig 29A and C).

In several experiments no antigen binding activity could be detected with kgH221A/gH341 or kgH221A/kgH341 combinations (see for example Fig 29), although expression levels were very low.

Antigen binding was detected when kgL221A/kgH341A or kgH221A/kgH341B combinations were expressed (Fig 29B). In the case of the antibody produced from the kgL221A/kgH341A combination the antigen binding was very similar to that of the chimaeric antibody (Fig 29B).

DISCUSSION

The objectives of the programme were to produce both a chimaeric mouse variable-human constant IgG4/K antibody and a fully humanised antibody retaining the antigen binding activity of the murine monoclonal antibody OKT3.

Cells were obtained from Ortho and mRNA prepared. A cDNA library was screened for heavy and light chain cDNAs using oligonucleotide probes. Full length cDNAs were obtained and the variable regions were sequenced (Figs 1 and 2). The cDNAs showed a high level of homology with sequences of antibodies which have specificity for alpha-1-6-dextran. It would be of interest to test OKT3 to determine whether it recognises and binds to dextran antigens.

The cDNAs were transferred to expression vectors (Figs 6 and 7) and expressed in COS cells. Antibody was produced which bound to an enriched T-cell population from peripheral blood cells.

Two versions of the chimaeric antibody were produced, differing in the light chain at the first amino acid of the constant region. In version 1 (Figs 9 and 13) the amino acid sequence which resulted at the V-C junction when the chimaeric light chain was constructed generates a potential N-linked glycosylation site at the 'elbow' region. This region is an extended sequence of peptide between the V and C domains and is potentially accessible to the enzymes of the glycosylation process. Fig 19 shows that the version 1 chimaeric light chain is glycosylated demonstrating that the secondary structure generated at the elbow is sufficient for the Asn-Arg-Thr motif to be used for glycosylation.

A second version of the chimaeric light chain was constructed (Fig 10) in which the first amino acid of the human constant region (Thr) was returned to the mouse amino acid (Ala), so removing the glycosylation site. Antibody was produced by coexpression with chimaeric heavy chain (Fig 13) and in both versions the chimaeric material was equivalent in binding to the mouse OKT3 (see Fig 19). These observations have been confirmed by Ortho staff (L Jolliffe pers. comm.).

Vectors for the expression of chimaeric OKT3 using neo/gpt or glutamine synthetase (GS) selection were prepared, including vectors in which both genes were on the same plasmid (Figs 15 to 18).

In the design of the fully humanised antibody the aim was to transfer the minimum number of mouse amino acids that would confer antigen binding onto a human antibody framework.

For the light chain the regions defining the loops known from

structural studies of other antibodies to contain the antigen contacting residues, and those hypervariable sequences defined by Kabat et al. as Complementarity Determining Regions (CDRs) are equivalent for CDR 2. For CDR1 the hypervariable region extends from residues 24-34 inclusive while the structural loop extends from 26-32 inclusive. In the case of OKT3 there is only one amino acid difference between the two options, at amino acid 24, where the mouse sequence is a serine and the human framework REI has glutamine. For CDR 3 the loop extends from residues 91-96 inclusive while the Kabat hypervariability extends from 89-97 inclusive. For OKT3 amino acids 89, 90 and 97 are the same between OKT3 and REI (Fig 20). When constructs based on the loop choice for CDR1 (gL121) and the Kabat choice (gL221) were made and coexpressed with mH or cH no evidence for antigen binding activity could be found for gL121, but trace activity could be detected for the gL221, suggesting that a single extra mouse residue in the grafted variable region could have some detectable effect. Both gene constructs were reasonably well expressed in the transient expression system.

The remaining framework residues were then further examined, in particular amino acids known from X-ray analysis of other antibodies to be close to the CDRs and also those amino acids which in OKT3 showed differences from the consensus framework for the mouse subgroup (subgroup VI) to which OKT3 shows most homology. Four positions, 1, 3, 46 and 47 were identified and their possible contribution was examined by substituting the mouse amino acid for the human amino acid at each position. Therefore gL221A (gL221 + D1Q, Q3V, L46R, L47W, see Fig 20 and Table 1) was made, cloned in EE6hCMVneo and coexpressed with cH (pJA144). The resultant antibody was well expressed and showed good binding activity (Fig 25 and Table 2). When the related genes gL221B (gL221 + D1Q, Q3V) and gL221C (gL221 + L46R, L47W) were made and similarly tested, while both genes produced antibody when coexpressed with cH, only the gL221C/cH combination showed good antigen binding (Fig 25). When the gL121A (gL121 + D1Q, Q3V, L46R, L47W) gene was made and coexpressed with cH antibody was produced which also bound to antigen (Fig 25).

For the heavy chain the loop and hypervariability analyses agree only in CDR3. For CDR1 the loop region extends from residues 26-32 inclusive whereas the Kabat CDR extends from residues 31-35 inclusive. For CDR2 the loop region is from 50-58 inclusive while the hypervariable region covers amino acids 50-65 inclusive. Therefore humanised heavy chains were constructed using the framework from antibody KOL and with various

combinations of these CDR choices, including a shorter choice for CDR2 of 50-56 inclusive as there was some uncertainty as to the definition of the end point for the CDR2 loop around residues 56 to 58. The genes were coexpressed with mL or cL initially. In the case of the gH genes with loop choices for CDR1 eg. gH121, gH131, gH141 very little antibody was produced in the culture supernatants (see Fig 27). As no free light chain was detected it was presumed that the antibody was being made and assembled inside the cell but that the heavy chain was aberrant in some way, possibly incorrectly folded, and therefore the antibody was being degraded internally. In some experiments trace amounts of antibody could be detected in ³⁵S labelling studies (see Fig 27). As no net antibody was produced, analysis of these constructs was not pursued further.

When, however, a combination of the loop choice and the Kabat choice for CDR1 was tested (mouse amino acids 26-35 inclusive) and in which residues 31 (Ser to Arg), 33 (Ala to Thr), and 35 (Tyr to His) were changed from the human residue to the mouse residue and compared to the first series, antibody was produced for gH321, kgH331 and kgH341 when coexpressed with cL (Fig 27). Expression was generally low and could not be markedly improved by the insertion of the Kozak consensus sequence 5' to the ATG of the signal sequence of the gene, as distinct from the case of the gL genes where such insertion led to a 2-5 fold increase in net antibody production (compare Figs 24 and 26). However, only in the case of gH341/mL or kgH341/cL could marginal antigen binding activity be demonstrated (see Fig 28 and Table 2). When the kgH341 gene was coexpressed with kgL221A, the net yield of antibody was too low (see Figs 29A column 6 and 29C laneE) to give a signal above the background level in the antigen binding assay (see Fig 29A column 5).

As in the case of the light chain the heavy chain frameworks were reexamined. Possibly because of the lower initial homology between the mouse and human heavy variable domains compared to the light chains, more amino acid positions proved to be of interest. Two genes, kgH341A and kgH341B were constructed, with 11 or 8 human residues respectively substituted by mouse residues compared to gH341, and with the CDR2 residue 63 returned to the human amino acid potentially to improve domain packing. Both showed good levels of expression with cL or kgL221A (Fig 29A) and both showed antigen binding with cL or kgL221A, the kgH341A gene with all 11 changes appearing to be the superior choice (Fig 29B).

It has been demonstrated here for OKT3 that to transfer antigen binding ability to the humanised antibody mouse residues outside the

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CDR regions defined by the Kabat hypervariability or structural loop choices are required for both the light and heavy chains. Fewer extra residues are needed for the light chain, possibly due to the higher initial homology between the mouse and human Kappa variable regions.

Of the changes seven (1 and 3 from the light chain and 6, 23, 71, 73 and 76 from the heavy chain) are predicted from a knowledge of other antibody structures to be either partly exposed or on the antibody surface. It has already been shown here that residues 1 and 3 in the light chain are not absolutely required to be the mouse sequence and for the heavy chain the gH341B heavy chain in combination with the 221A light chain generates activity without the presence of the 6 and 23 changes. It would be of interest to determine by further study the individual contribution of the other 8 mouse residues of the kgH341A gene compared to kgH341 and to determine whether the 7 extra mouse surface residues in the antibody produced by the kgH341A/kgL221A combination contribute to idiotypic epitopes which can be detected by sera from patients treated with murine OKT3.

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1 GAATTCCCAA AGACAAATG GATTTCAGG TGGAGATTG GAGCTTCCTG

51 CTAATCAGTG CCTCAGTCA AATAACAGA GGACAAATTG TTCTCACCCT

101 GTCTCCAGCA ATCATGTCTG CATTCTCAGG GGAGAAGGTC ACCATTGACCT

151 GCAGTGCCAG CTCAAGTGTA AGTTACATGA ACTGGTACCA GCAGAAGTCA

201 GGCACCTCCC CCAAAAGATG GATTATGAC ACATCCAAAC TGGCTTCTGG

251 AGTCCCTGCT CACTTCAGGG GCAGTGGGTC TGGGACCTCT TACTCTCTCA

301 CAATCAGCGG CATGGAGGCT GAAGATGCTG CCACCTATTA CTGCCAGCAG

351 TGGAGTAGTA ACCCATTCACT GTTCGGCTCG GGGACAAAGT TGGAAATAAA

401 CCGGGCTGAT ACTGCACCAA CTGTATCCAT CTTCCCACCA TCCAGTGAGC

451 AGTTAACATC TGGAGGTGCC TCAGTCGTGT GCTTCTTGAA CAACTTCTAC

501 CCCAAAGACA TCAATGTCAA GTGGAAGATT GATGGCAGTG AACGACAAAA

551 TGGCGTCCTG AACAGTTGGA CTGATCAGGA CAGCAAAGAC AGCACCTACA

601 GCATGAGCAG CACCCTCAGT TTGACCAAGG ACGAGTATGA ACGACATAAC

651 AGCTATACCT GTGAGGCCAC TCACAAGACA TCAACTTCAC CCATTGTCAA

701 GAGCTTCAAC AGGAATGAGT GTTAGAGACA AAGGTCCTGA GACGCCACCA

751 CCAGCTCCCA GCTCCATCCT ATCTTCCCTT CTAAGGTCTT GGAGGCTTCC

801 CCACAAGCGC TTACCACTGT TGCGGTGCTC TAAACCTCCT CCCACCTCCT

851 TCTCCTCCTC CTCCCTTTCC TTGGCTTTTA TCATGCTAAT ATTTGCAGAA

901 AATATTCAAT AAAGTGAGTC TTTGCCTTGA AAAAAAAAAA AAA

SEQUENCE LENGTH 943 RESIDUES

INITIATOR MET AT 18

MATURE SEQUENCE BEGINS AT 84

CODING SEQUENCE 639 RESIDUES

NB. KAPPA CHAIN SEQUENCE OBTAINED FROM PUBLISHED SEQUENCE.
ONLY THE JUNCTION WITH VARIABLE REGION AND 3' UNTRANSLATED REGION
HAS BEEN CHECKED.

OKT3 LIGHT CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA SEQUENCE

1 MDFOVOIESF LLISASVIIS RQIVLTQSP AIMSASPGEK VTMTCSASSS

51 VSYMNWYQQK SGTSPKRWIY DTSKLAGVP AHFRGSGSGT SYSLTISGME

101 AEDAATYYCQ QWSSNPFTFG SGTKLEINRA DTAPTVSIFP PSSEQLTSGG

151 ASVVCFLNNE YPKDINVKWK IDGSRQNGV LNSWTDQDSK DSTYSMSSTL

201 TLTKDEYERH NSYTCEATHK TSTSPIVKSF NRNEC*

FIGURE 1

The DNA sequence of the OKT3 light chain as deduced from DNA sequencing of cDNA's and, for the Kappa constant region, from known sequence.

Untranslated regions are shown in uppercase type and the signal sequence is underlined. Also shown is the protein sequence translated from the major open reading frame.

1 GAATTCCCCT CTCCACAGAC ACTGAAACT CTGAGTCAC AGGAGG
51 ACTGGAATCTT TCTGCTCTTG TCTGCTCTTG CTGAGGCTT CCACTGACAG
101 GTCCAGCTGC AGCAGTCTGG GGCTGAACTG GCAAGACCTG GGGCCTCACT
151 GAAGATGTCC TGCAAGGCTT CTGGCTACAC CTTTACTAGG TACACGATGC
201 ACTGGGTAAA ACAGAGGCCT GGACAGGGTC TGGAAATGGAT TGGATACATT
251 AATCCTAGCC GTGGTTATAC TAATTACAAT CAGAAGTTCA AGGACAAGGC
301 CACATTGACT ACAGACAAAT CCTCCAGCAC AGCCTACATG CAACTGAGCA
351 GCCTGACATC TGAGGACTCT GCAGTCTATT ACTGTGCAAG ATATTATGAT
401 GATCATTACT GCCTTGACTA CTGGGGCCAA GGCACCACTC TCACAGTCTC
451 CTCAGCCAAA ACAACAGCCC CATCGGTCTA TCCACTGGCC CCTGTGTGTG
501 GAGATACAAC TGGCTCCTCG GTGACTCTAG GATGCCTGGT CAAGGGTTAT
551 TTCCCTGAGC CAGTGACCTT GACCTGGAAC TCTGGATCCC TGTCCAGTGG
601 TGTGCACACC TTCCCAGCTG TCCTGCAGTC TGACCTCTAC ACCCTCAGCA
651 GCTCAGTGAC TGTAACCTCG AGCACCTGGC CCAGCCAGTC CATCACCTGC
701 AATGTGGCCC ACCCGGCAAG CAGCACCAAG GTGGACAAGA AAATTGAGCC
751 CAGAGGGCCC ACAATCAAGC CCTGTCCTCC ATGCAAATGC CCAGCACCTA
801 ACCTCTTGGG TGGACCATCC GTCTTCATCT TCCCTCCAAA GATCAAGGAT
851 GTACTCATGA TCTCCCTGAG CCCCATAGTC ACATGTGTGG TGGTGGATGT
901 GAGCGAGGAT GACCCAGATG TCCAGATCAG CTGGTTTGTG AACAACGTGG
951 AAGTACACAC AGCTCAGACA CAAACCCATA GAGAGGATTA CAACAGTACT
1001 CTCCGGGTGG TCAGTGCCCT CCCCATCCAG CACCAGGACT GGATGAGTGG
1051 CAAGGAGTTC AAATGCAAGG TCAACAACAA AGACCTCCCA GCGCCCATCG
1101 AGAGAACCAT CTCAAAACCC AAAGGGTCAG TAAGAGCTCC ACAGGTATAT
1151 GTCTTGCTC CACCAGAAGA AGAGATGACT AAGAAACAGG TCACTCTGAC
1201 CTGCATGGTC ACAGACTTCA TGCCTGAAGA CATTTACGTG GAGTGGACCA
1251 ACAACGGGAA AACAGAGCTA AACTACAAGA AACTGAACC AGTCCTGGAC
1301 TCTGATGGTT CTTACTTCAT GTACAGCAAG CTGAGAGTGG AAAAGAAGAA
1351 CTGGGTGGAA AGAAATAGCT ACTCCTGTTC AGTGGTCCAC GAGGGTCTGC
1401 ACAATCACCA CACGACTAAG AGCTTCTCCC GGACTCCGGG TAAATGAGCT
1451 CAGCACCCAC AAAACTCTCA GGTCCAAAGA GACACCCACA CTCATCTCCA
1501 TGCTTCCCTT GTATAAATAA AGGACCCAGC AATGCCTGGG ACCATGTAAA
1551 AAAAAAAAAA AAAGGAATTC

SEQUENCE LENGTH 1570 RESIDUES

INITIATOR MET AT 41

SIGNAL SEQUENCE UNDERLINED

MATURE SEQUENCE BEGINS AT 98

CODING SEQUENCE 1407 RESIDUES

5' UNTRANSLATED REGION 40 RESIDUES 3' UNTRANSLATED REGION 123
RESIDUES

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3
35OKT3 HEAVY CHAIN PROTEIN SEQUENCE DEDUCED FROM DNA
SEQUENCE

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.1 MERHWIFLLL LSVTAGVHSQ VQLQQSGAEL ARPGASVKMS CKASGYTFTTR
51 YTMHWVKQRP GQGLEWIGYI NPSRGYTNYN QKFKDKATLT TDKSSSTAYM
101 QLSSLTSEDS AVYYCARYYD DHYCLDYWGQ GTTLTVSSAK TTA²PSVYPLA
151 PVCGDTTGSS VTLGCLVKGY FPEPVTLTWN SGSLSSGVHT FPAVLQSDLY
201 TLSSSVTVTS STWPSQSITC NVAHPASSTK VDKKIEPRGP TIKPCPPCKC
251 PAPNLLGGPS VFIFPPKIKD VLMISLSPIV TCVVVDVSED D²PDVQISWFV
301 NNVEVHTAQT QTHREDYNST LRVVSALPIQ HQDWMSGKEF KCKVNNKDLP
351 APIERTISKP KGSVRAPQVY VLPPPEEEMT KKQVTLTCMV TDFMPEDIYV
401 EWTNNGKTEL NYKNTEPVLD SDGSYFMYSK LRVEKKNWVE RNSYSCSVVH
451 EGLHNHHTTK SFSRTPGK*

FIGURE 2

The DNA sequence of the OKT3 heavy chain chain as deduced from DNA sequencing of cDNA's and, for the constant regions, from known sequence.

The signal sequence is underlined.

Also shown is the protein sequence translated from the major open reading frame.

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$$\begin{array}{r} 4 \\ \hline 35 \end{array}$$

```

99  SGTKLEINR 107
    . | | | | |
101 AGTKLELKR 109

```

HOMOLOGY 92.5%

UPPER LINE OKT3 V_L

LOWER LINE MOUSE V_L SUB GROUP 6

FIGURE 3

The protein sequence comparison of the OKT3 light chain variable region with the Kabat mouse sub group 6 consensus sequence (Kabat et al. 1987).

```

      OKT3 QVQLQQSGAELARPGASVKMSCKASGYTFTRYTMHWVKQRPGQGLEWIGY 50
            |||||  | | | | | | | | | | | | | | | | | | | | | | | |
5A  CONS evqlqqsgaelvragssvkmsckasgytftsyglnwvkqrpgqglewigy 50
            .
      51 INPSRGYTNYNQKFQKDKATLTDDKSSSTAYMQLSSLTSEDSAVYYCAR.. 98
            |||  |||  ||  |||  ||  || | | | | | | | | | | | | | | | |
      51 inpgngytkynekfkgkttltvdkssstaymqlrsltsedsavyfcarsn 100
            .
      99 YYDDHYC..LDYWGQGTTTLTVSS 119
            ||  |  || | | | | | | | | |
     101 yyggsyyffffdywgggttltvss 123      Percent Similarity: 81.513

```

The protein sequence comparison of the OKT3 heavy chain variable region with the Kabat mouse sub groups 2A, 2B, 2C and 5A (Kabat et al. 1987)

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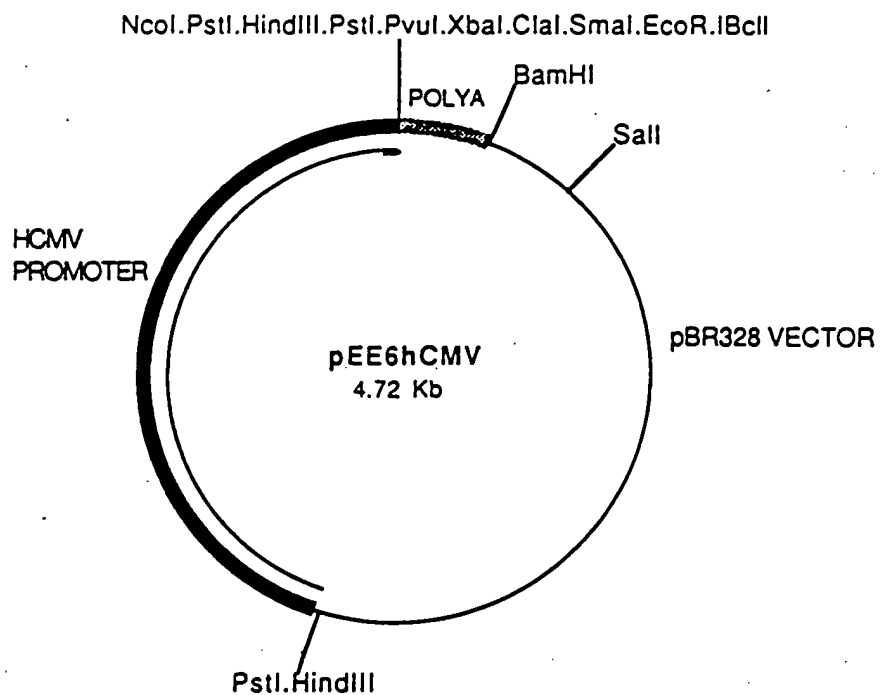


FIGURE 5

A map for the EE6hCMV expression vector used in this study
Only necessary sites are shown.

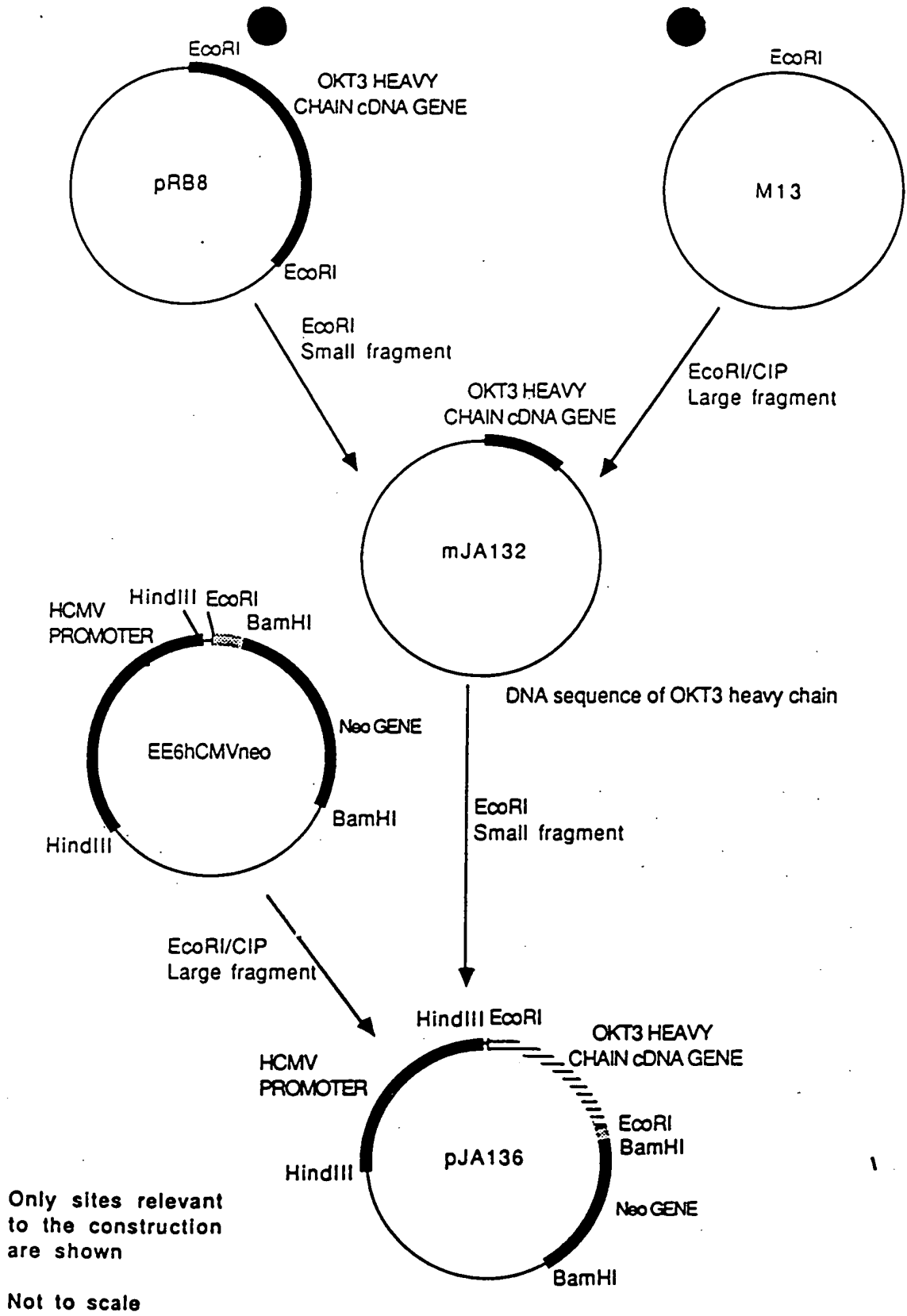


FIGURE 6
An outline schematic of the procedures involved in the construction of pJA136, a vector for the expression in eukaryotic cells of the OKT3 heavy chain cDNA gene.

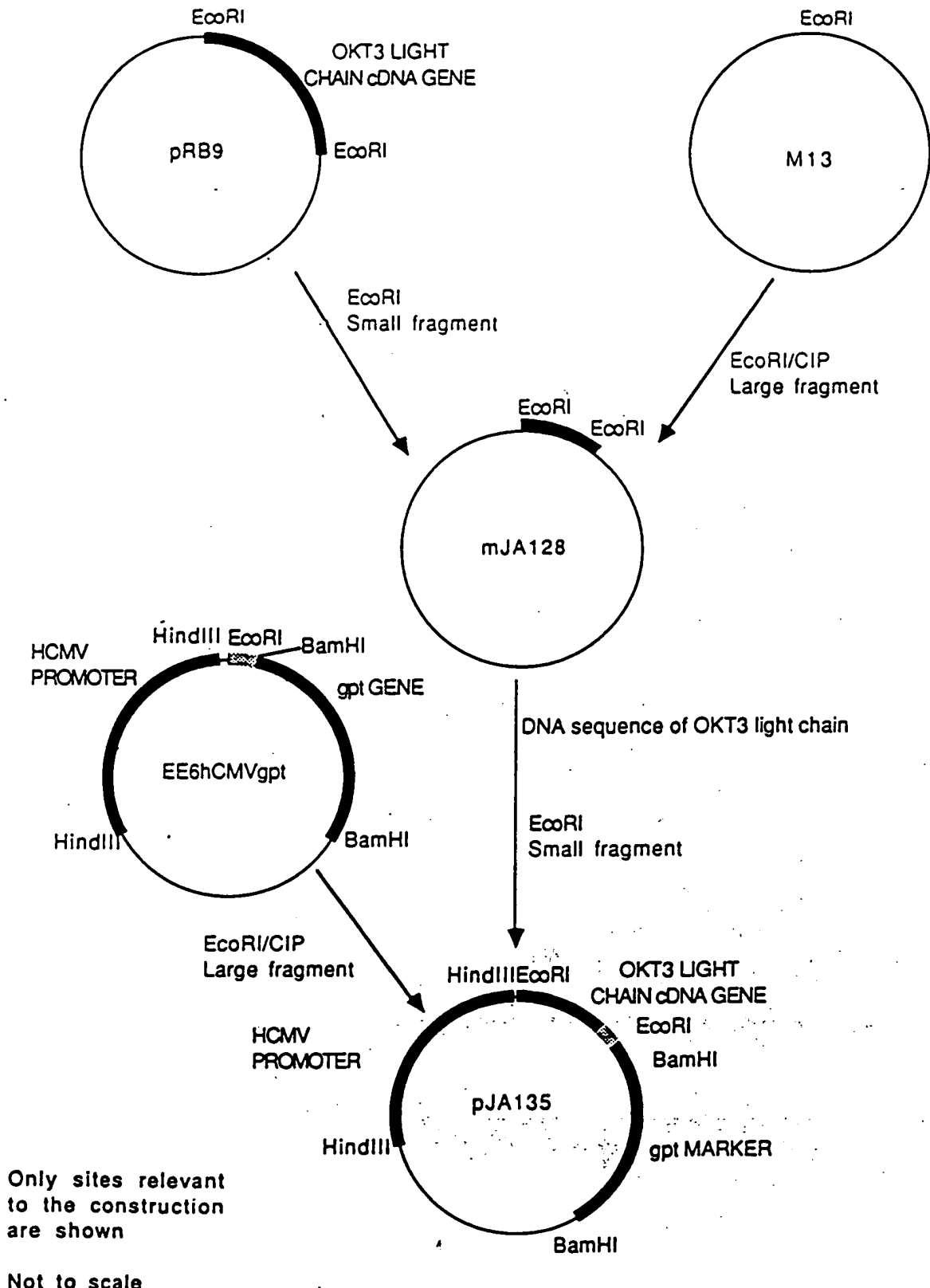


FIGURE 7

An outline schematic of the procedures involved in the construction of pJA135, a vector for the expression in eukaryotic cells of the OKT3 light chain cDNA gene.

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E
C
O
R
I
gaattcccaaagacaaaATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATCAGTG
1 -----+-----+-----+-----+-----+-----+ 60
CCTCAGTCATAATATCCAGAGGAcaaattgttctcaccagtcctccagcaatcatgtctg
61 -----+-----+-----+-----+-----+-----+ 120
catctccaggggagaaggtcaccatgacctgcagtgccagctcaagtgttaagttacatga
121 -----+-----+-----+-----+-----+-----+ 180
actggtaccagcagaagtcaggcacctccccaaaagatggatttatgacacatccaaac
181 -----+-----+-----+-----+-----+-----+ 240
tggcttctggagtcacctgctcacttcaggggcagtgggctctgggacctcttactctctca
241 -----+-----+-----+-----+-----+-----+ 300
caatcagcggcatggaggctgaagatgctgccacttattactgccagcagtgaggtagta
301 -----+-----+-----+-----+-----+-----+ 360
A
v
a
I
acccattcacgttcggctcggggacaaagttggaataaaccgg
361 -----+-----+-----+-----+-----+-----+ 404

FIGURE 8

The nucleotide sequence for the OKT3 light chain variable region and the location of the EcoRI and Aval sites used in the construction of the chimaeric OKT3 light chain gene.

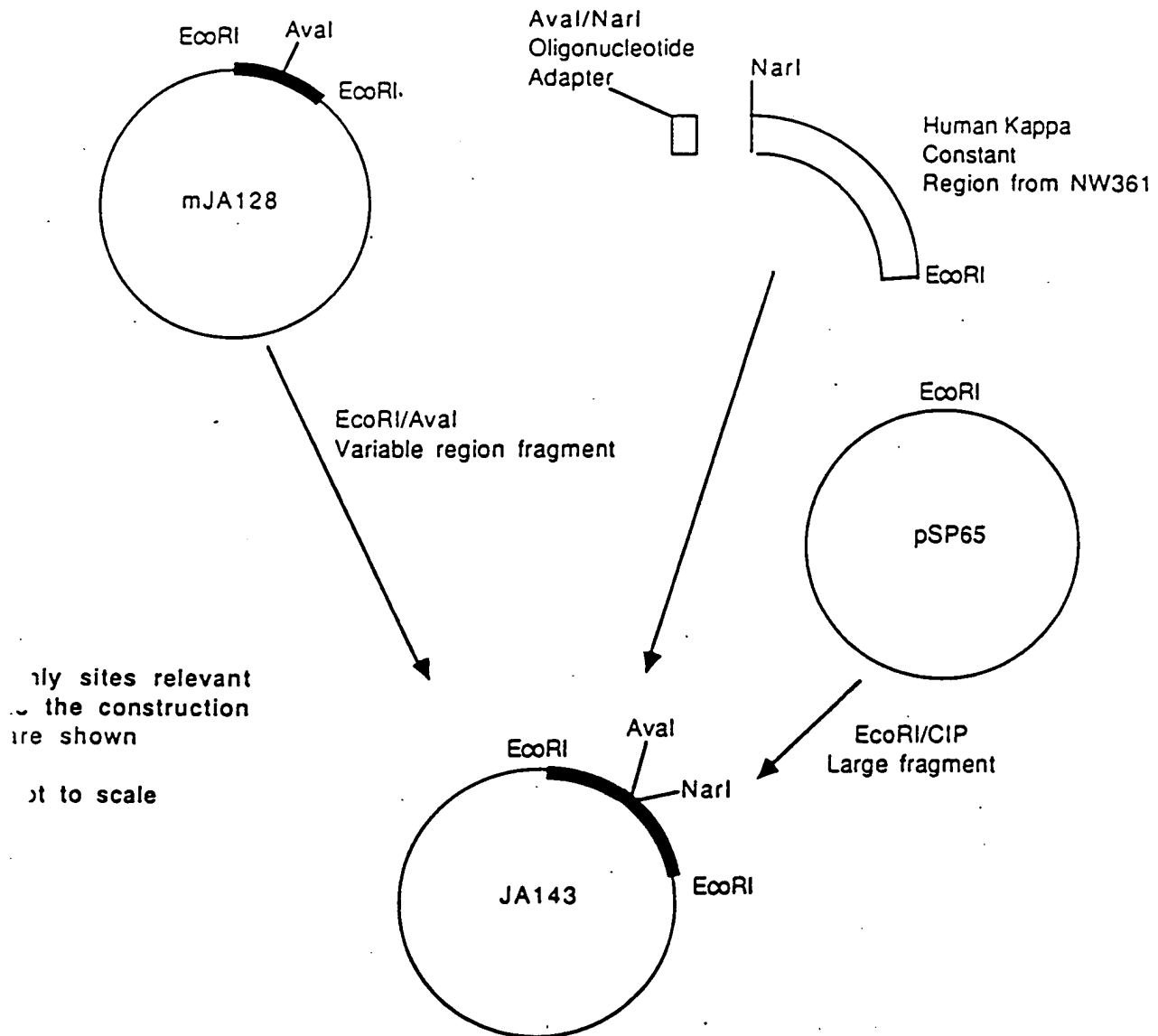


FIGURE 9

An outline schematic of the procedures involved in the construction of pJA143, an M13 vector, including the OKT3 chimaeric light chain gene (Version1).

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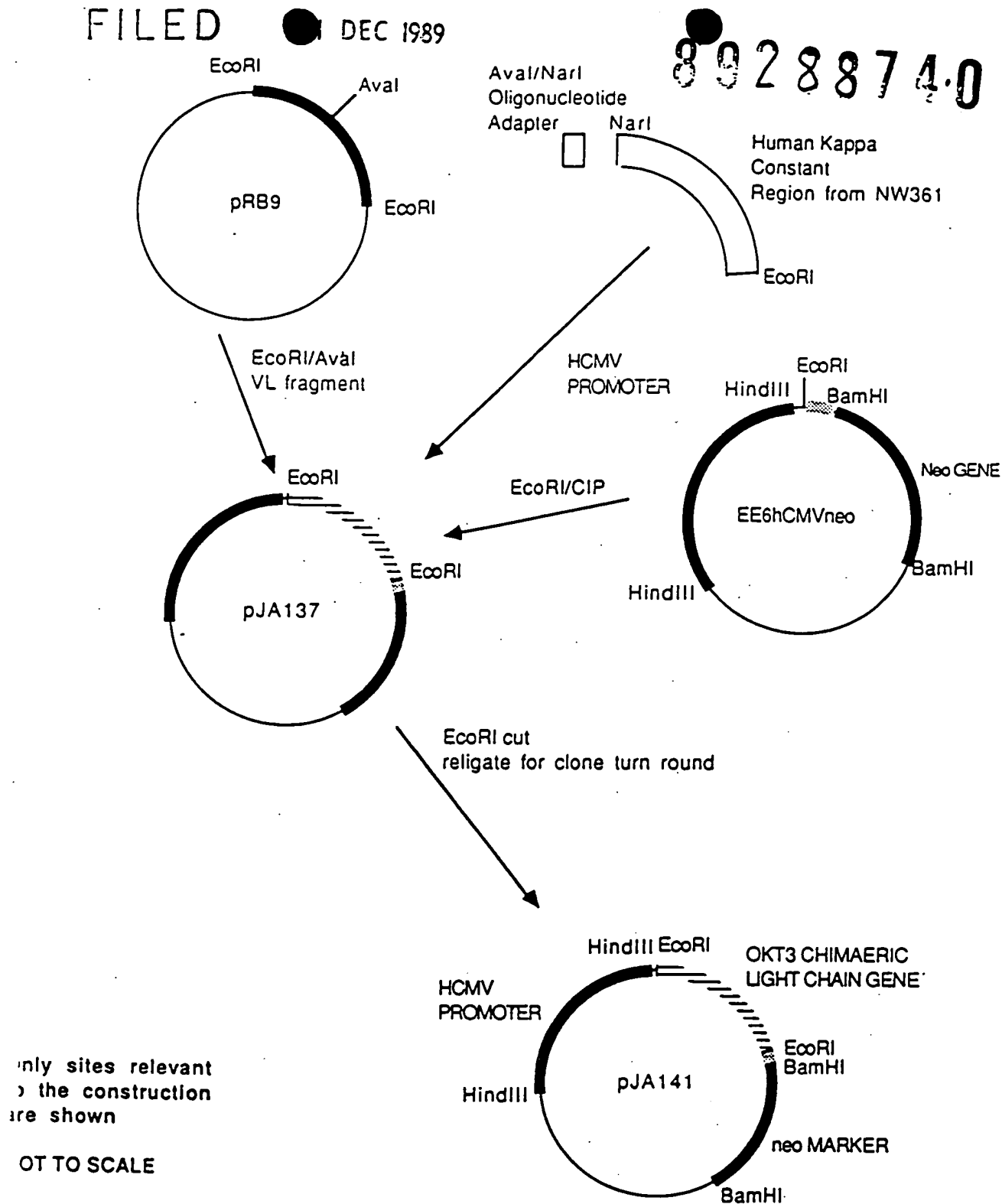


FIGURE 10

An outline schematic of the procedures involved in the construction of pJA141, a vector for the expression in eukaryotic cells of the OKT3 chimaeric light chain gene (Version 2).

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E
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GAATTCCTCTCCACAGACACTGAAACTCTGACTCAACATGGAAAGGCACTGGATCTT
-----+-----+-----+-----+-----+-----+ 60

61 TCTACTCCTGTTGTCAGTAACTGCAGGTGTCCACTCCCAGGTCCAGCTGCAGCAGTCTGG
-----+-----+-----+-----+-----+-----+ 120

121 GGCTGAACTGGCAAGACCTGGGGCCTCAGTGAAGATGTCCTGCAAGCTTCTGGCTACAC
-----+-----+-----+-----+-----+-----+ 180

181 CTTTACTAGGTACACGATGCACTGGGTAAAACAGAGGCCTGGACAGGGTCTGGAATGGAT
-----+-----+-----+-----+-----+-----+ 240

241 TGGATACATTAATCCTAGCCGTGGTTATACTAATTACAATCAGAAGTTCAAGGACAAGG
-----+-----+-----+-----+-----+-----+ 300

301 CACATTGACTACAGACAAATCCTCCAGCACAGCCTACATGCAACTGAGCAGCCTGACATC
-----+-----+-----+-----+-----+-----+ 360

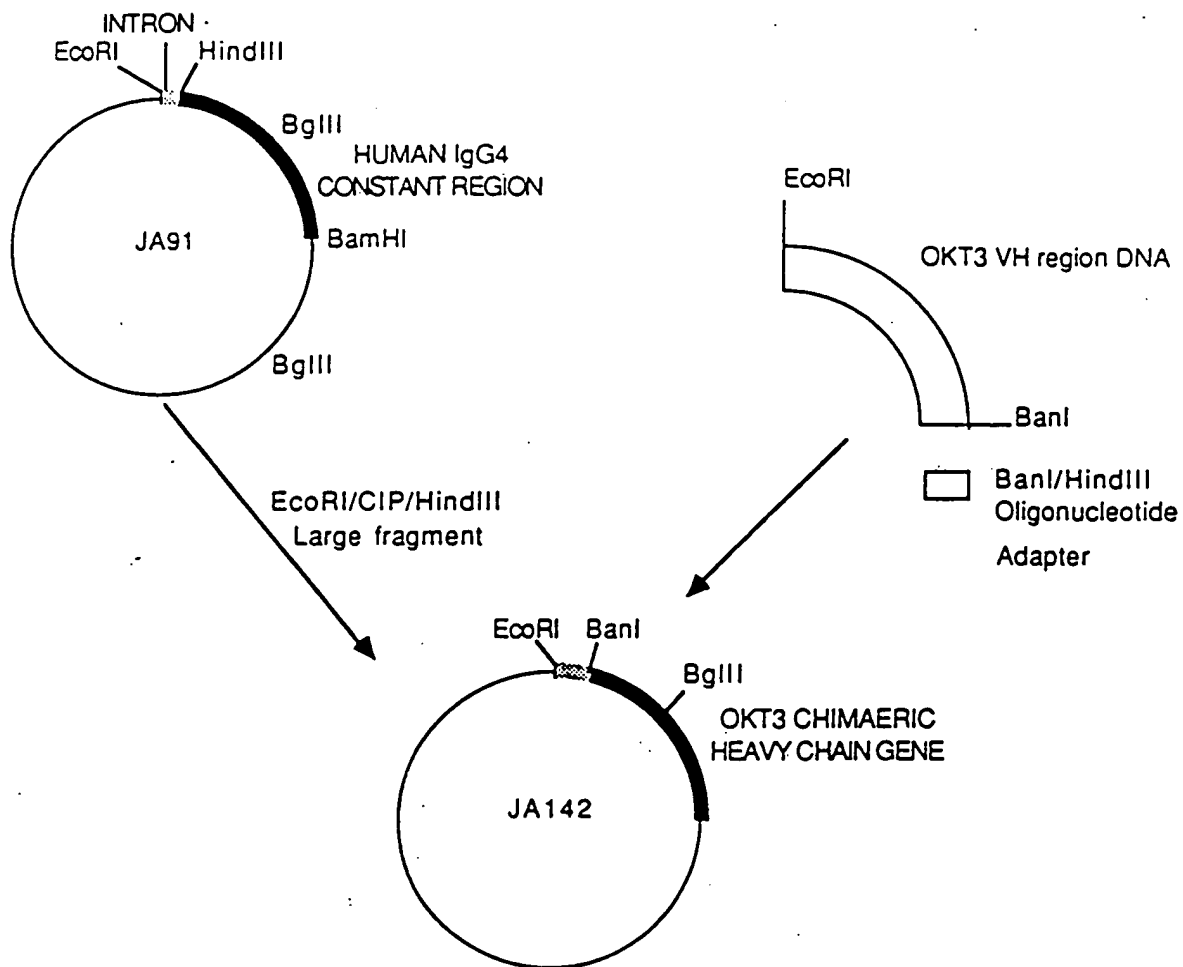
361 TGAGGACTCTGCAGTCTATTACTGTGCAAGATATTATGATGATCATTACTGCCTTGACTA
-----+-----+-----+-----+-----+-----+ 420

B
a
n
I

421 CTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
-----+-----+-----+-----+-----+ 454

FIGURE 11

The nucleotide sequence for the OKT3 heavy chain variable region and the location of the EcoRI and BanI sites used in the construction of the chimaeric OKT3 heavy chain gene.



Only sites relevant
to the construction
are shown

FIGURE 12

An outline schematic of the procedures involved in the construction of pJA142, an M13 vector including the OKT3 chimaeric heavy chain gene.

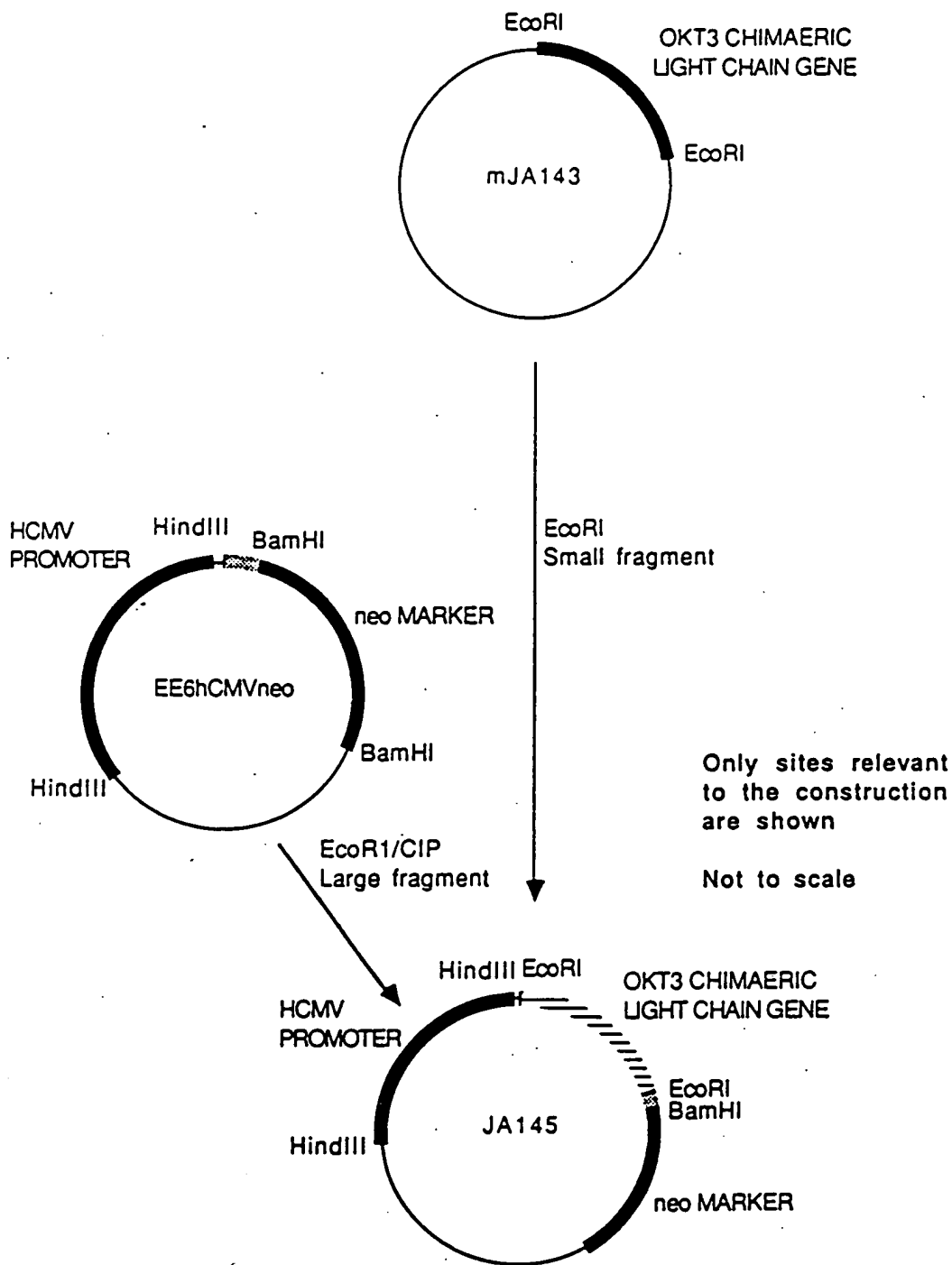


FIGURE 13

An outline schematic of the procedures involved in the construction of pJA145, a vector for the expression in eukaryotic cells of the OKT3 chimaeric light chain gene (Version 1).

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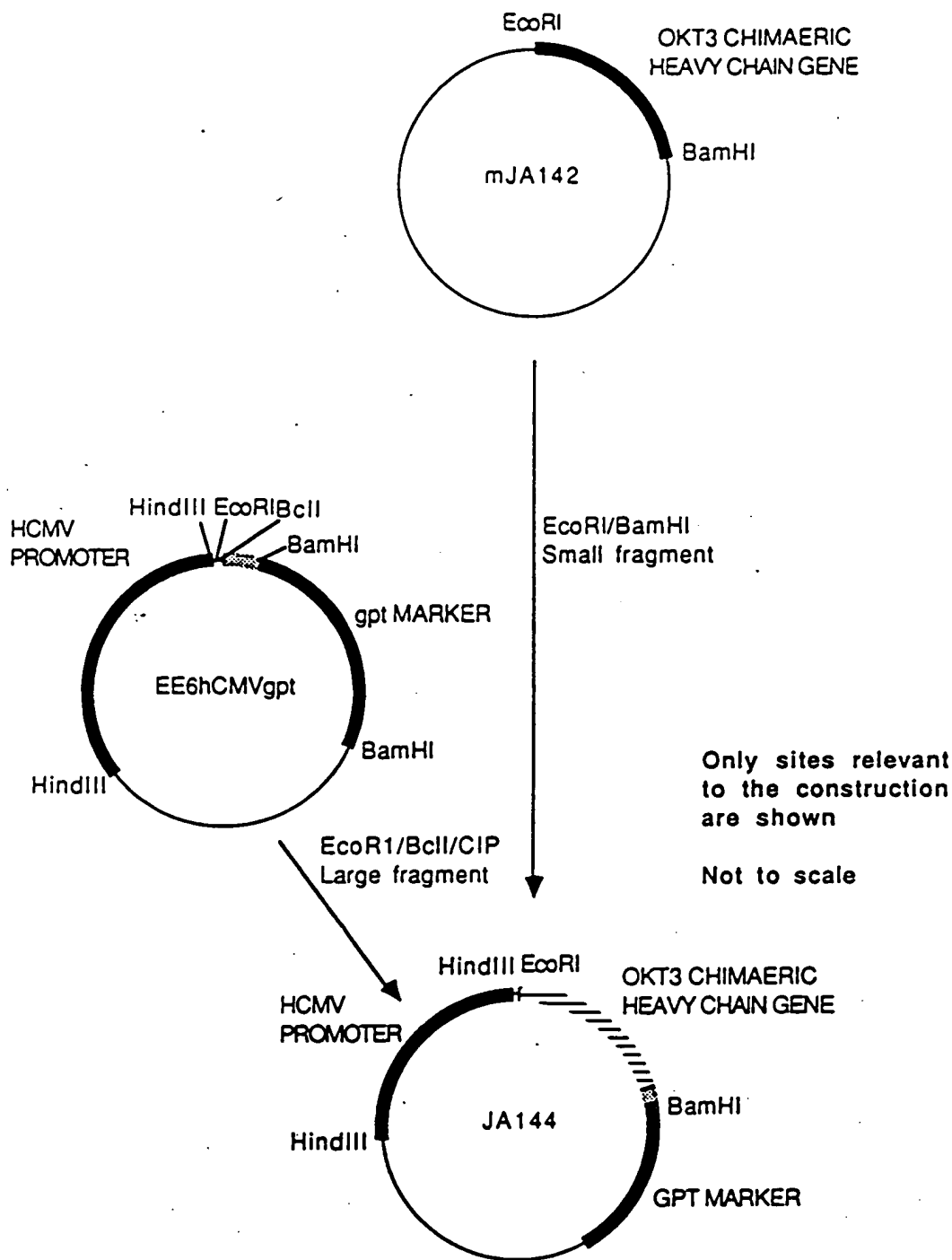


FIGURE 14

An outline schematic of the procedures involved in the construction of pJA144, a vector for the expression in eukaryotic cells of the OKT3 chimaeric heavy chain gene.

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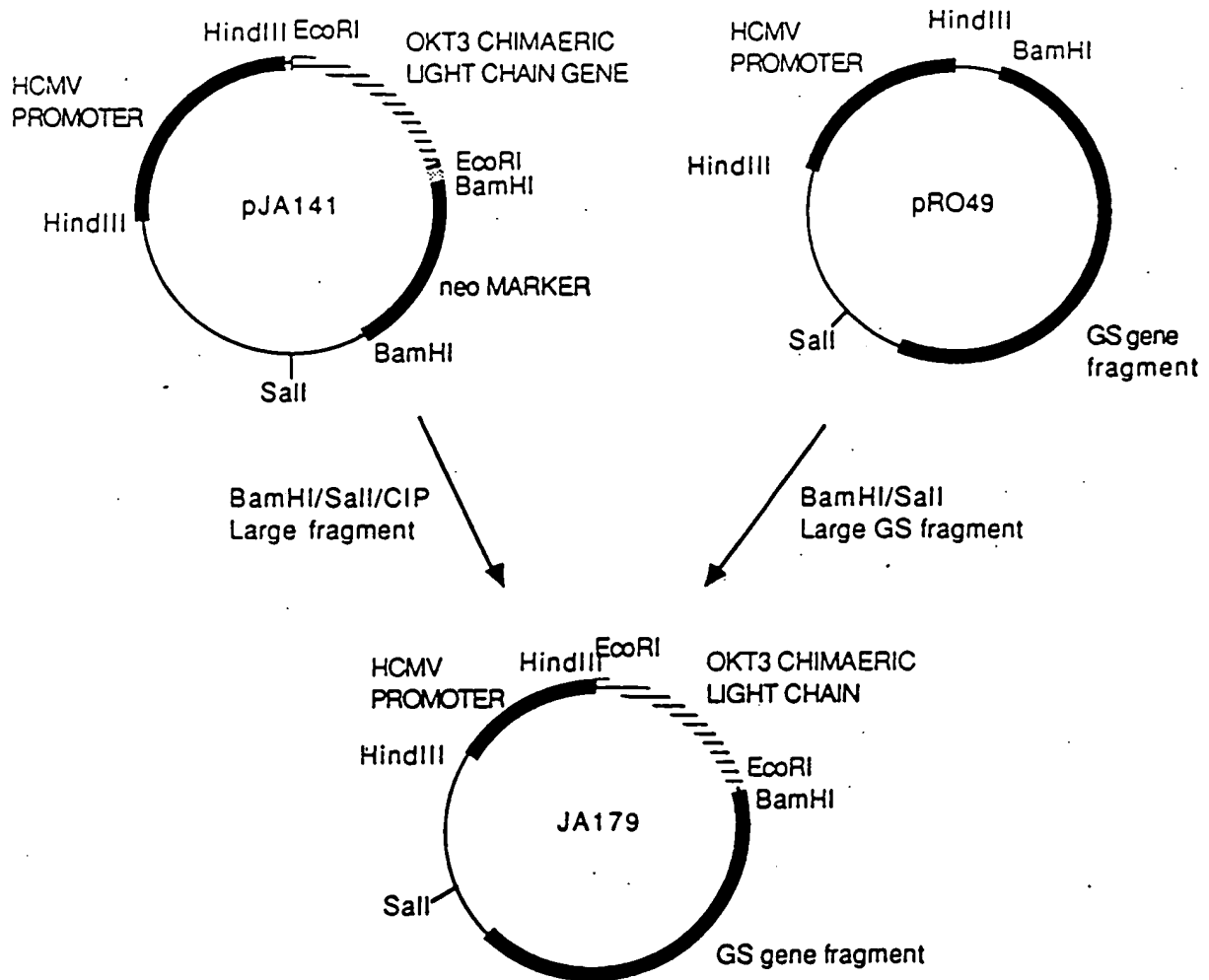


FIGURE 15

An outline schematic of the procedures involved in the construction of pJA179, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric light chain gene (Version 2).

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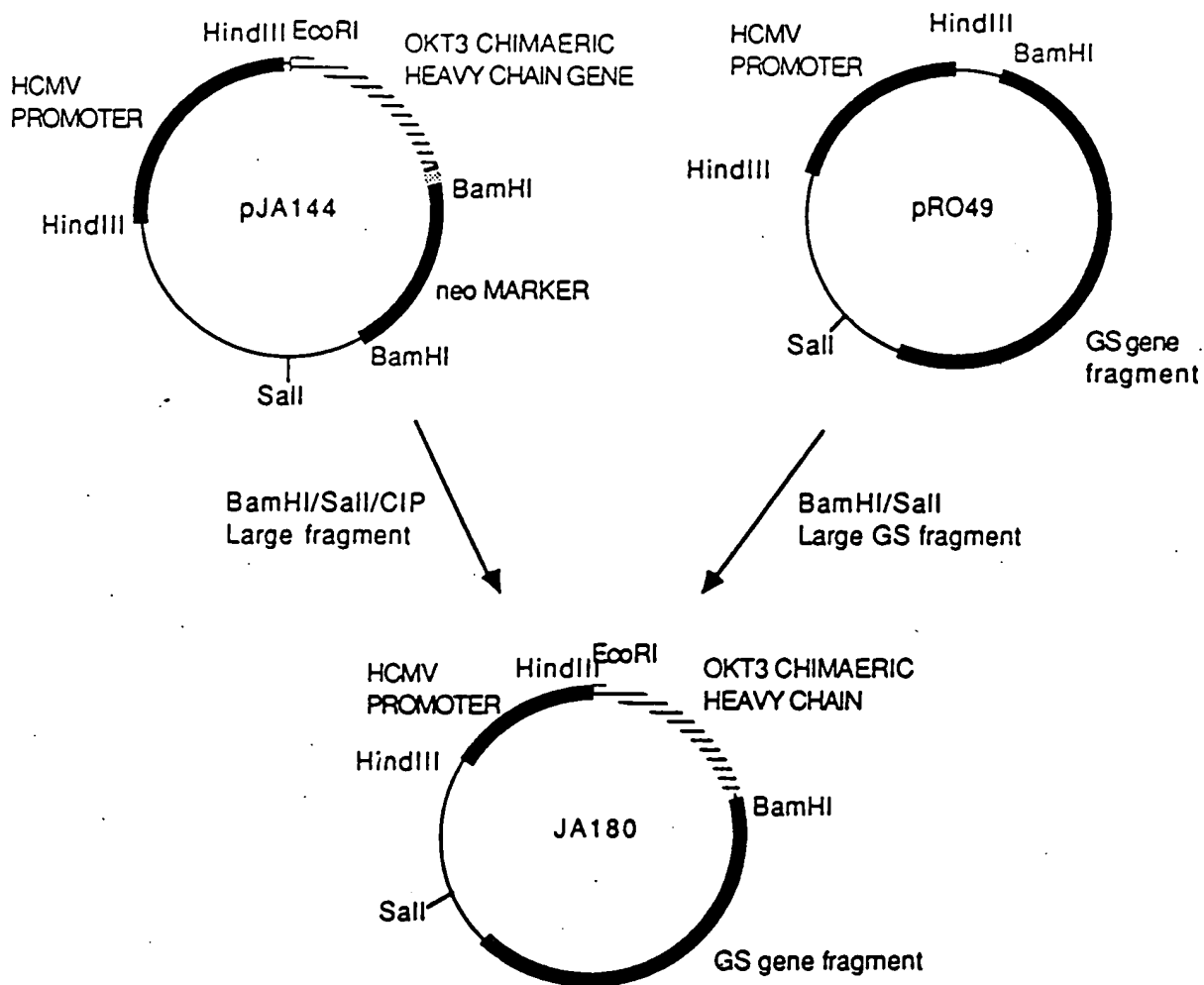


FIGURE 16

An outline schematic of the procedures involved in the construction of pJA179, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric heavy chain gene.

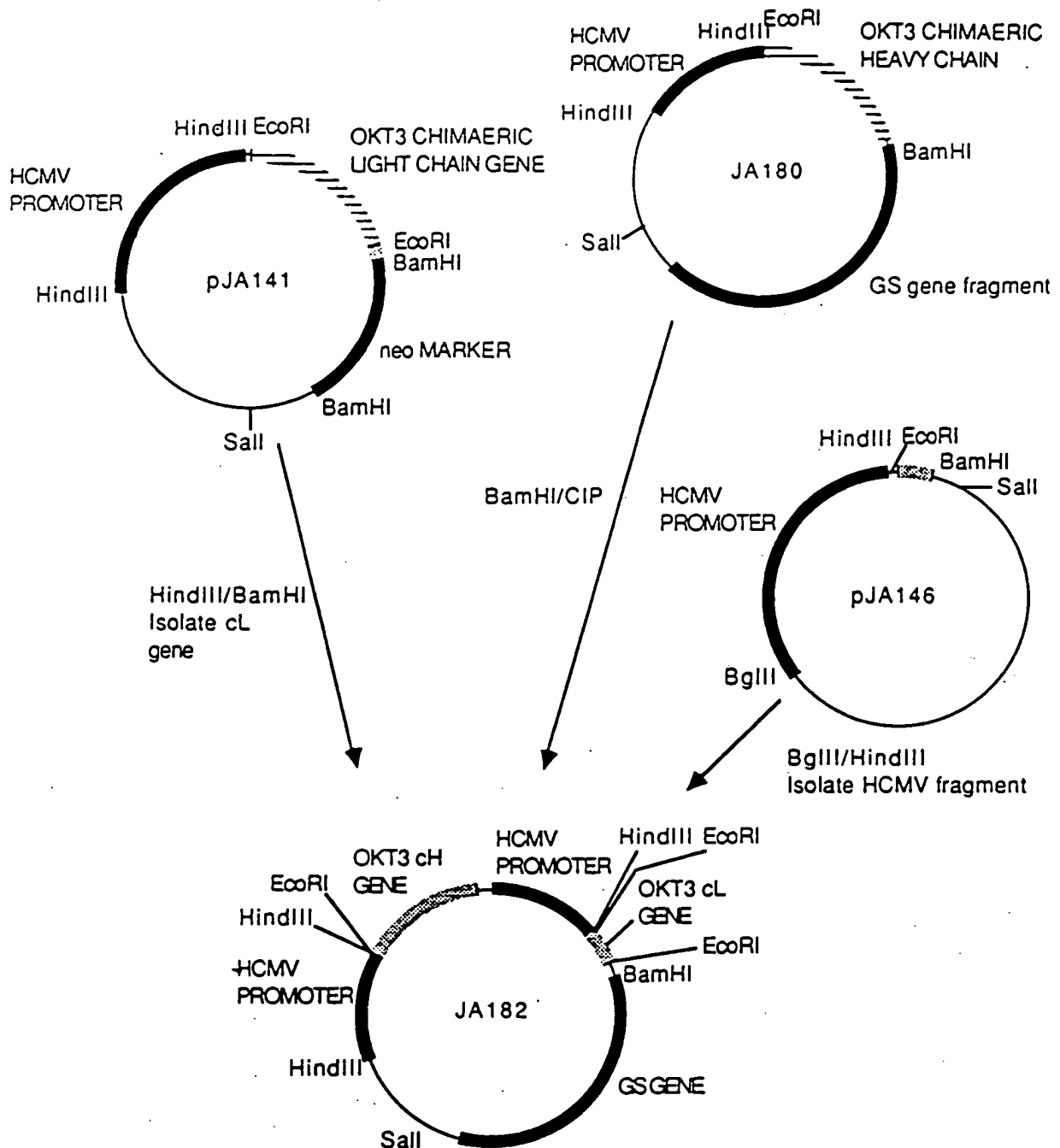


FIGURE 17

An outline schematic of the procedures involved in the construction of pJA182, a vector for the expression in eukaryotic cells using the GS amplification system of the OKT3 chimaeric heavy chain gene and chimaeric light chain gene (Version 2) in the transcription order cH>cL>GS.

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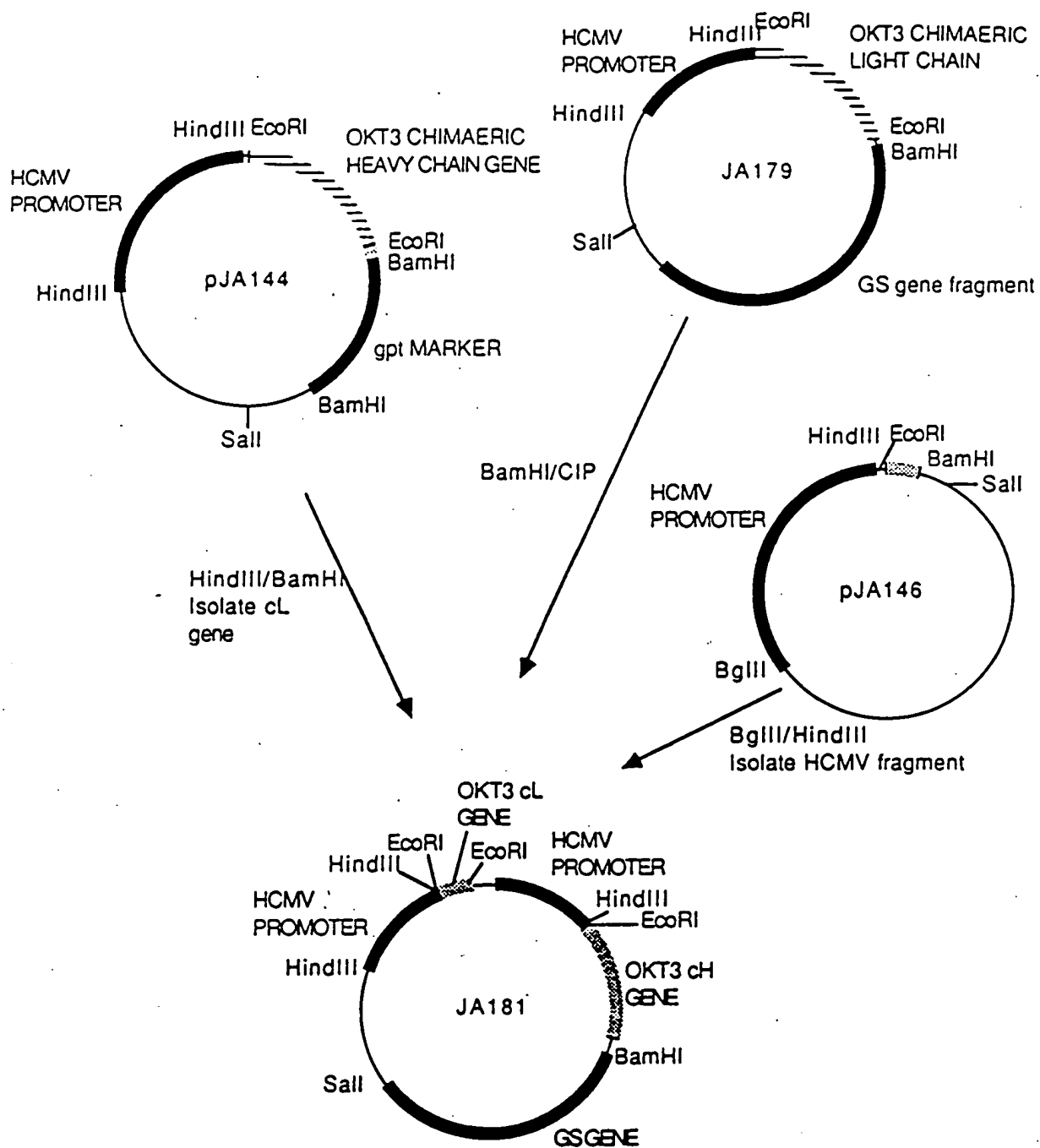
19
35

FIGURE 18

An outline schematic of the procedures involved in the construction of pJA181, a vector for the expression in eukaryotic cells using the GS amplification system, of the OKT3 chimaeric light chain gene (Version 2) and chimaeric heavy chain gene in the transcription order cL>cH>GS.

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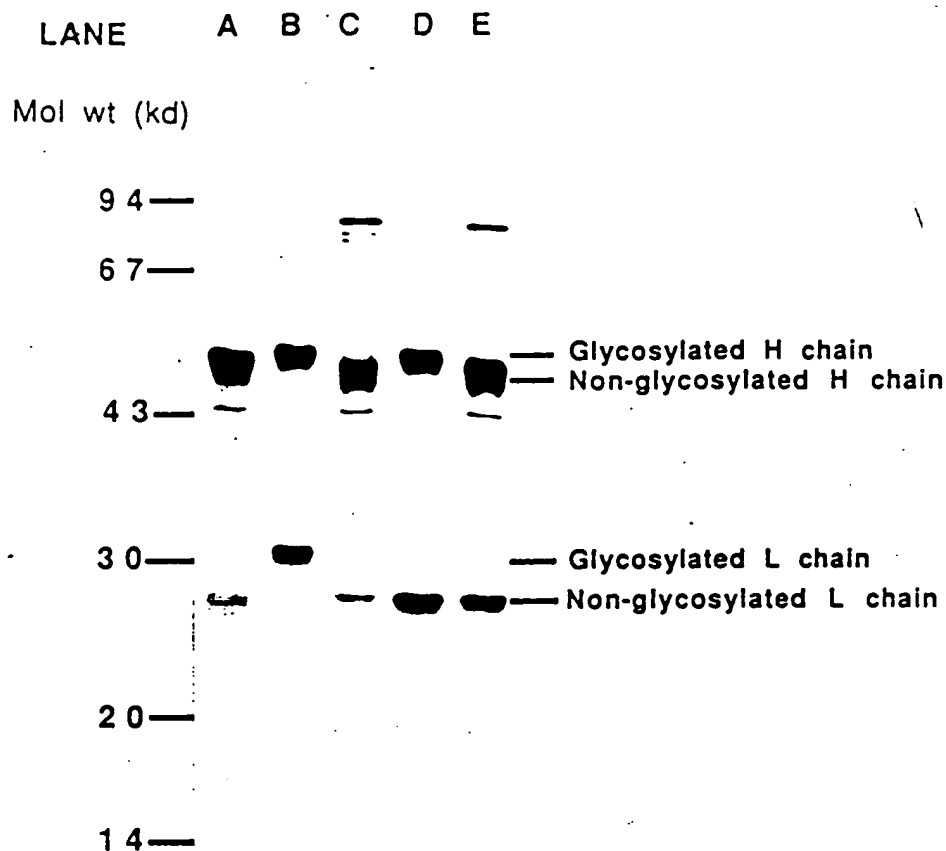


Fig 19. Effect on glycosylation of the presence of tunicamycin during cell growth

Reducing SDS-PAGE of ^{35}S labelled antibody produced in the absence (lanes A, B, D) or presence (lanes C & E) of tunicamycin. COS cells were transfected and medium replaced after 24hrs by medium with or without tunicamycin. Antibody was recovered from culture supernatants by protein-A Sepharose precipitation. after 48hrs further incubation.

Key:

- A. - cLcH B72.3 control
- B. - cL*cH - Tunicamycin
- C. - cL*cH + Tunicamycin
- D. - cLcH - Tunicamycin
- E. - cLcH + Tunicamycin

NB: cL* - chimaeric light chain version 1
cL - chimaeric light chain version 2

21
35

23 42
NN N N N N
RES TYPE SBspSPESsssBSbSsssPSPSPsPSsse*s*p*Pi*ISsSe
Okt3v1 QIVLTQSPAIMSASPGEKVTMTCSASS.SVSYNNWYQQKSGT
REI DIQMTQSPSSLSASVGDRTITCQASQDI IKYLNWYQQTPGK
? ?
CDR1 (LOOP) *****
CDR1 (KABAT) *****

56

85

NN
RES TYPE *IsiPpIeesesssSBesePsPSBSSEsPspPsseesSPePb
Okt3v1 SPKRWIYDTSKLASGVPAHFRGSGSGTSYSLTISGMEAEDAAT
REI APKLLIYEASNLOAGVPSRFSGSGSGTDYFTFTISSLPEDIAT
? ?? ? ?
***** CDR2 (LOOP/KABAT)

102 108

RES TYPE PiPIPIes**iPIIsPPSPSPSS
Okt3v1 YYCQQWSSNPFTFGSGTKLEINR
REIv1 YYCQQYQSLPYTFGQGTKLQITR
? ?
***** CDR3 (LOOP)
***** CDR3 (KABAT)

KEY TO RES TYPE

N NEAR TO CDR (FROM X RAY STRUCTURES)

P PACKING

B BURIED NON PACKING

S SURFACE

E EXPOSED

I INTERFACE

* INTERFACE/PART EXPOSED

^ PACKING/PART EXPOSED

? NON-CDR RESIDUES WHICH MAY REQUIRE TO BE LEFT AS MOUSE SEQUENCE

FIGURE 20

The alignment of the OKT3 light variable region amino acid sequence with that of the light variable region of the human antibody REI. Above the sequence the residue type (defined in the key) notes the spatial location of each residue side chain (derived by examination of resolved structures from Xray crystallography analysis). Residues in bold type refer to amino acids which differ from the residue found at that position in the consensus sequence for the species subgroup to which the antibody belongs ie. mouse sub group 6 for the OKT3 sequence and human sub group 1 for the REI sequence.

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```

NN N                23 26      32 35 N39  43
RES TYPE  SESPs^SBsss^sSSsSpSpSPsPSEbSBsssBePiPiPiesss
Okt3vh    QVQLQQSGAELARPGASVFMSCKASGYTFTRYTMHWVKQRPGQ
KOL        QVQLVESGGGVVQPGRSLRLSCSSSGFIFSSYAMYWVRQAPGK

```

?

??

***** CDR1 (LOOP)

***** CDR1 (KABAT)

```

52a      60 65      N N N      82abc      89
RES TYPE  IIeIppp^ssssssss^ps^pSSsbSpseSsSseSp^pSpSBssS^ePb
Okt3vh    GLEWIGYINPSRGYTNYNQKFKDKATLTDDKSSSTAYMQLSSLTSEDSAV
KOL        GLEWVAIIWDDGSDQHYADSVKGRFTISRDN SKNTLFLQMDSLRPEDTGV

```

??

? ? ? ?

?

***** CDR2 (LOOP)

***** CDR2 (KABAT)

```

92 N                107      113
RES TYPE  PiPIEissssiisssbibi*EIPIP*spSBSS
Okt3vh    YYCARYYDDHY.....CLDYWGQGTTLTVSS
KOL        YFCARDGGHGFCSSASCFGPDYWGQGPVTVSS

```

***** CDR3 (KABAT/LOOP)

KEY TO RES TYPE

N NEAR TO CDR (FROM X RAY STRUCTURES)

P PACKING

B BURIED NON PACKING

S SURFACE

E EXPOSED

I INTERFACE

* INTERFACE/PART EXPOSED

^ PACKING/PART EXPOSED

? AMINO ACIDS WHICH MAY NEED TO REMAIN AS MOUSE IN CDR GRAFT

FIGURE 21

The alignment of the OKT3 heavy variable region amino acid sequence with that of the heavy variable region of the human antibody KOL. Above the sequence the residue type (defined in the key) notes the spatial location of each residue side chain (derived by examination of resolved structures from Xray crystallography analysis). Residues in bold type refer to amino acids which differ from the residue found at that position in the consensus sequence for the species subgroup to which the antibody belongs ie. mouse sub group 2B for the OKT3 sequence and human sub group 3 for the KOL sequence.

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```

1  AATTCATGGA ATGGAGCTGG GTCTTTTCTCT TCTTCCTGTC AGTAACTACA
51 GGTGTCCACT CCCAGGTTCA GCTGCTGGAG TCTGAGGAG GAGTCGTCCA

                                     26 27 28 29 30
                                     G  Y  T  F  T
101 GCCTGGAAGG TCCCTGAGAC TGTCTTGTTT TTCTTCTGGA TACACATTCA
                                     oligo JA88-44 cct atgtgtaagt...
                                     PROBE JA88-45
                                     G  Y  T  F

31 32 33 34 35
D  H  A  M  Y  W
151 CAGACCACGC TATGTACTGG GTCAGACAGG CTCCTGGAAA GGGACTGGAG
    **** *
...gttctatgtg atacgtgacc cagtctgtcc 5' R1198
gttctatgtg atacgt 5' R1197
T  R  Y  T  M  H  W

50 51 52 53 54 55 56 57 58 59 60 61 62
Y  I  S  P  G  N  D  D  I  K  Y  N  E  K
201 TGGGTCGCTT ACATCTCTCC TGGAAATGAC GACATCAAGT ACAATGAGAA
    *** * * * * *
...acccagcgaa tgtaattagg atcgtctcct atgtgtttta tgtagtctt...
    PROBE JA88-41 gg atcgtctcct atgtgttta 5' R1153
    Y  I  N  P  S  R  G  Y  T  N  Y  N  Q  K

63 64 65 66
F  K  G  R
251 GTTCAAGGGA AGATTCACAA TTTCTAGAGA CAATTCTAAG AATACACTGT
    **
...caagttcctg tctaagtgtt aaagatc 5' R1152
    F  K  D  R

301 TCCTGCAGAT GGACTCACTC AGACCTGAGG ACACAGGAGT CTACTTCTGT
                                     oligo JA88-42 tgaagaca

95 96 97 98 99 100 a b 101102
S  Y  Y  G  H  D  Y
351 GCTAGATCCT ACTACGGCCA C..... GACTACTGGG GCCAAGGTAC
    * * * * *
cgatctatga tgctgctggt gatgacagac ctgatgaccc cgggtt 5' R1154
PROBE JA88-43 a tgctgctggt gatg 5' R1155
    Y  Y  D  D  H  Y  C  L  D  Y

401 CCCGGTCACC GTGAGCTC

```

KEY

LINE 1 AMINO ACID SEQUENCE NUMBERS (KABAT NOMENCLATURE)
 LINE 2 AMINO ACID SEQUENCE OF B72.3 GH341V_H REGION (PARENT)
 LINE 3 NUCLEOTIDE SEQUENCE OF JA148 (B72.3 GH341 PARENT SEQ)
 LINE 4 * LOCATION OF POINT MUTATIONS
 LINE 5 NUCLEOTIDE SEQUENCE OF MUTAGENIC OLIGONUCLEOTIDES
 LINE 6 NUCLEOTIDE SEQUENCE OF PROBE OLIGONUCLEOTIDES
 LINE 7 AMINO ACID SEQUENCE OF MUTATED SEQUENCE (GH341 OKT3)

FIGURE 22A

The DNA sequence of the B72.3 grafted heavy (GH341) sequence (J Adair and A Docherty unpublished) and the sequences of oligonucleotides necessary to replace the CDR regions with OKT3 CDRs and to act as specific probes for the desired alterations.

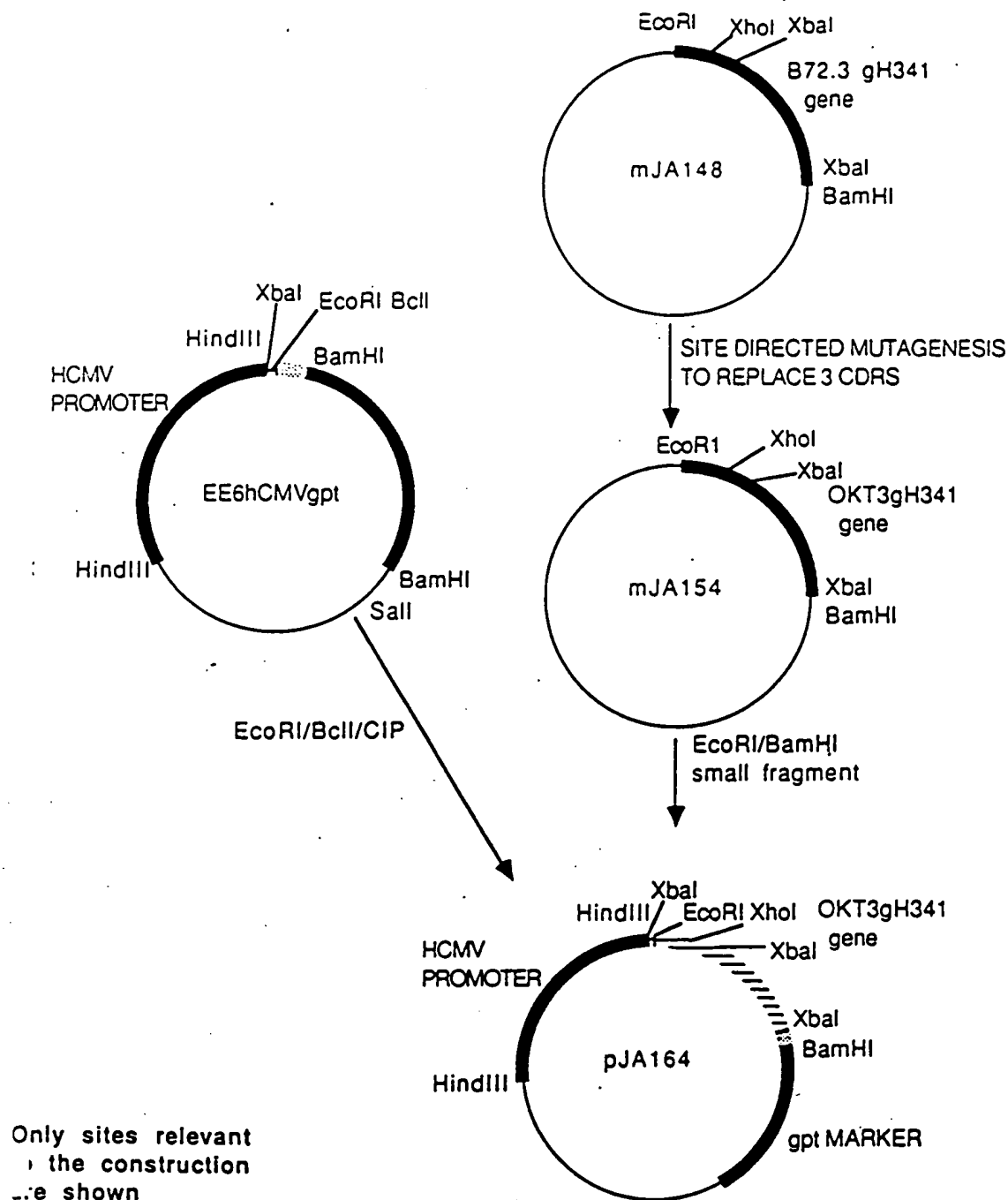


FIGURE 22B
An outline schematic of the procedures involved in the construction of pJA164, a vector for the expression in eukaryotic cells of the OKT3 gH341 CDR grafted heavy chain gene.

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ACZAK SEQ SIGNAL SEQ

M E X S V F L F F L S Y

R1387 1 AATTGGCGGGCACCGTGGAAATGGAGGTGGGCTTTCTTTTCTTCCTGTGAGTA 31

R1388 2 GCGGCGGTGGTACCTTACCTCGACCCAGAGAGAAGAAGGAC 41

MATURE V₂

		T	T	G	V	H	S	Q	V	Q	L	V	
R1090	3	<u>ACTACAGGTGTCCACTCCAGGTTTCAGCTGGTG</u>											33
R1091	4	AGTCATTGATGTCCACAGGTGAGGGTCCAAGTC											33

		q	S	G	G	G	V	V	Q	P	G	
R1594	5	CAGTCTGGAGGAGGAGTCGTCCAGCCTGGA										30
R1595	6	GACCACqTCAGACCTCCTCCTCAGCAGGTC										30

		R	S	L	R	L	S	C	k	a	
R1540	7	AGGTCCCTGAGACTGTCTTGTaaggct									27
R1095	8	GGACCTTCCAGGGACTCTGACAGAACA									27

			S	G	Y	T	F	T	R	Y	T	M	H	CDR1
R1385	9		T	C	T	G	G	A	T	A	C	A	C	33
R1590	10	ttccga	A	G	A	C	T	A	T	G	T	G	G	45

		W V R Q A P G K G L E W i	
R1591	11	TGGGTCAGACAGGCTCCTGGAAAGGGACTCGAGTGGatt	39
R1258	12	TCTGTCCGAGGACCTTTCCCTGAGCTC	27
		-XhoI-	

```

                g Y I N P S R G Y T N Y
R1586 13      ggaTACATTAATCCTAGCAGAGGTTATACTAACTAC
R1587 14      ACCtaacctATGTAATTAGGATCGTCTCCAATATGATTGATG

```

N Q K v K D R	CDR2
AATCAGAAGgtgAAGGACAGA	57
TTAGTCTTCcactTCCTGTCTAAGTGT	69

		F T I S t D k S K s T a	
R1599	15	TTCACAATTTCTactGACaaaTCTAAGagcACAgcc	36
R1600	16	TAAAGAtgaCTGtttAGATTctcg	24

		F	L	Q	M	D	S	L	R	P	
R1106	17	TTCCTGCAGATGGACTCACTCAGACCT	27								
R1601	18	TGTcggAAGGACGTCTACCTGAGTGAG	27								

		E D T a V Y y C A	
R1680	19	GAGGATACCGccGTCTAtTatTGTGCT	27
R1681	20	TCTGGACTCCTATGGCggCAGATaAta	27

		R Y Y D D H Y C L D Y W	CDR3
R1426	21	AGATATTACGATGACCACTACTGTCTGGACTACTGG	36
R1427	22	ACACGATCTATAATGCTACTGGTGATGACAGACCTGATGACCCCGGTT	48

```
G Q G T P V T V S S a
R1114 23 GGCCAAGGTACCCCGGTCACCGTGAGCTC 29
R1115 24 CCATGGGGCCAGTGGCACTCGAGTCGA 27
```

>CH1 DOMAIN

FIGURE 23A

The sequences of oligonucleotides necessary to construct, by oligonucleotide assembly procedures, the OKT3 CDR grafted kgH341A gene. Above the nucleotide sequences are shown the peptide sequences coded by the oligonucleotides. Lower case nucleotide and amino acid residues show differences compared to the gH341 sequence (see Fig 22A).

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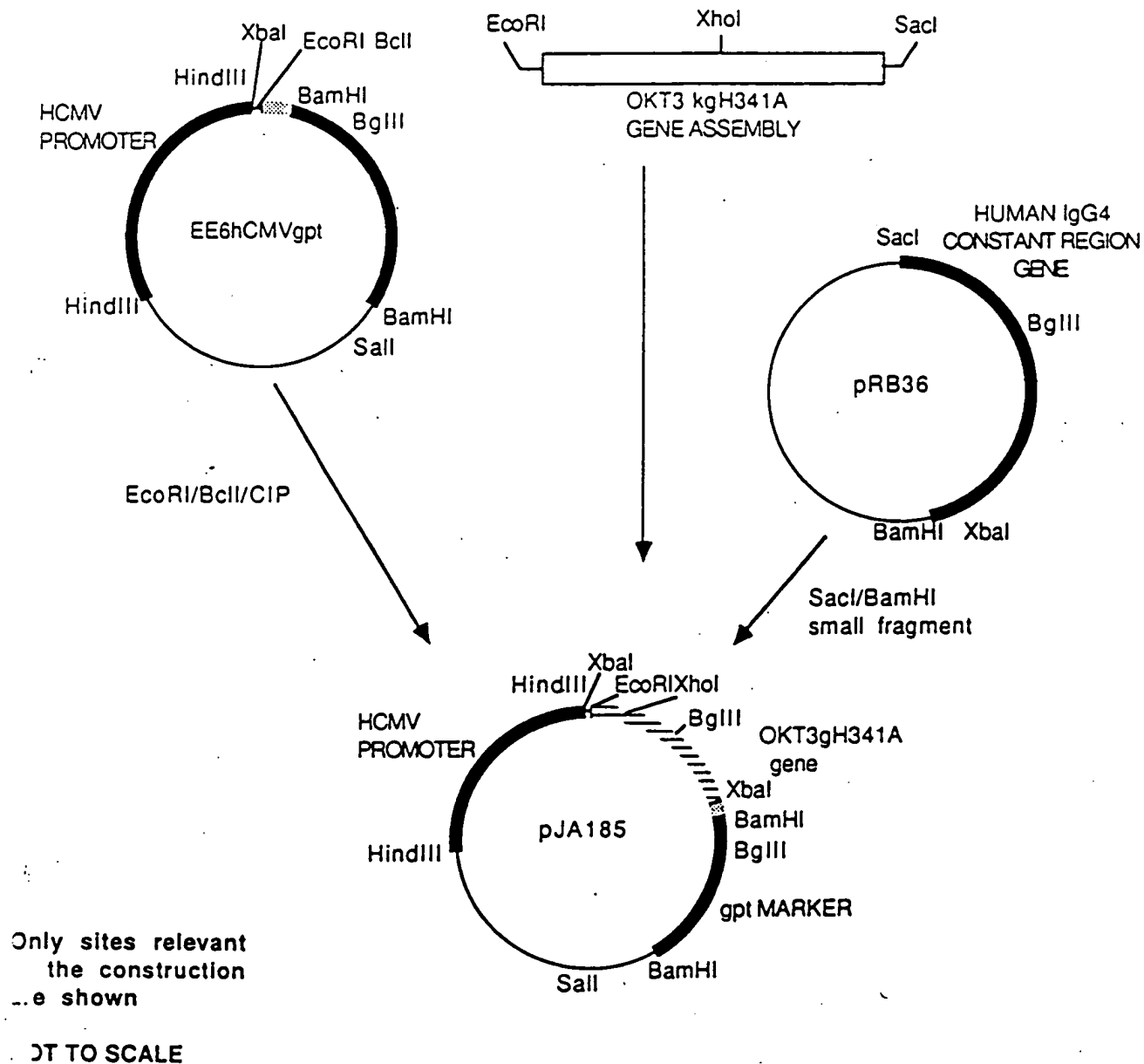


FIGURE 23B
An outline schematic of the procedures involved in the construction of pJA185,
a vector for the expression in eukaryotic cells of the OKT3
gH341 CDR grafted heavy chain gene.

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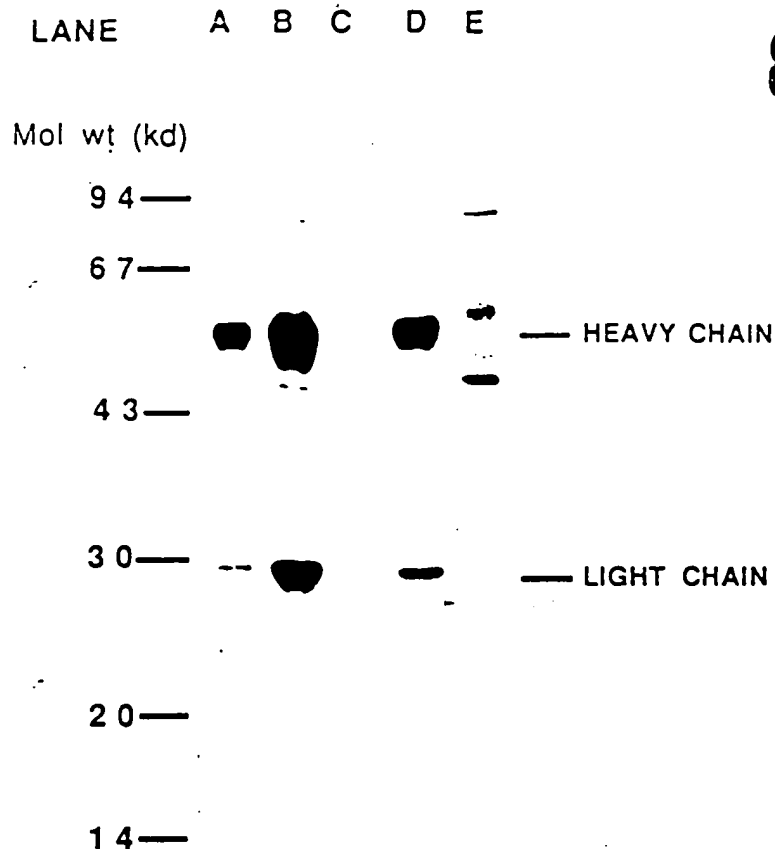


Fig 24a. Effect on antibody expression of the presence of a "Kozak consensus sequence" immediately preceding the gL gene.

Reducing SDS-PAGE of ^{35}S labelled antibody purified from a COS cell transient expression experiment by Protein A-Sepharose precipitation.

KEY:

- A. - gL221 cH
- B. - gLK221 cH
- C. - gL221A cH
- D. - gLK221A cH
- E. - Mock transfection

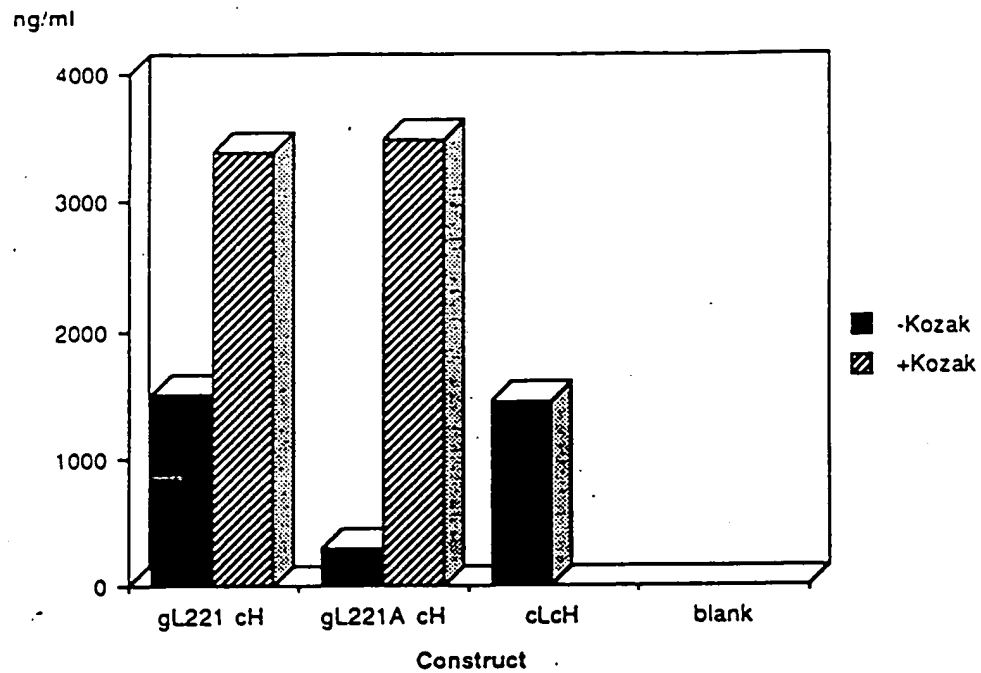


Fig 24b. Effect on antibody expression in the presence of the Kozak consensus sequence immediately preceding the gL gene.

Yield of antibody (ng/ml) from COS cell transient expression experiment.

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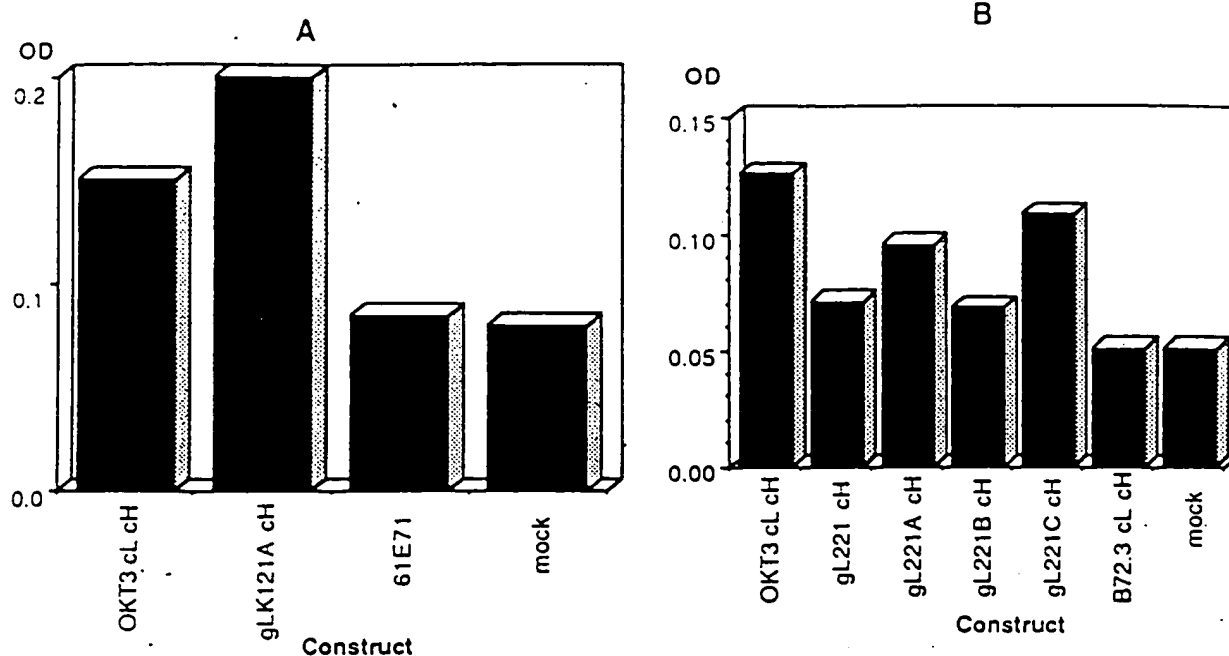
29
35

Fig 25. Antigen binding data for gL series genes

Culture supernatants from COS cell transient expression experiments. Various combinations of gL and cH genes were tested for binding to Hut 78 cells.

Chimaeric B72.3 or chimaeric 61E71 was used as a negative control

For codes to genes see table 1.

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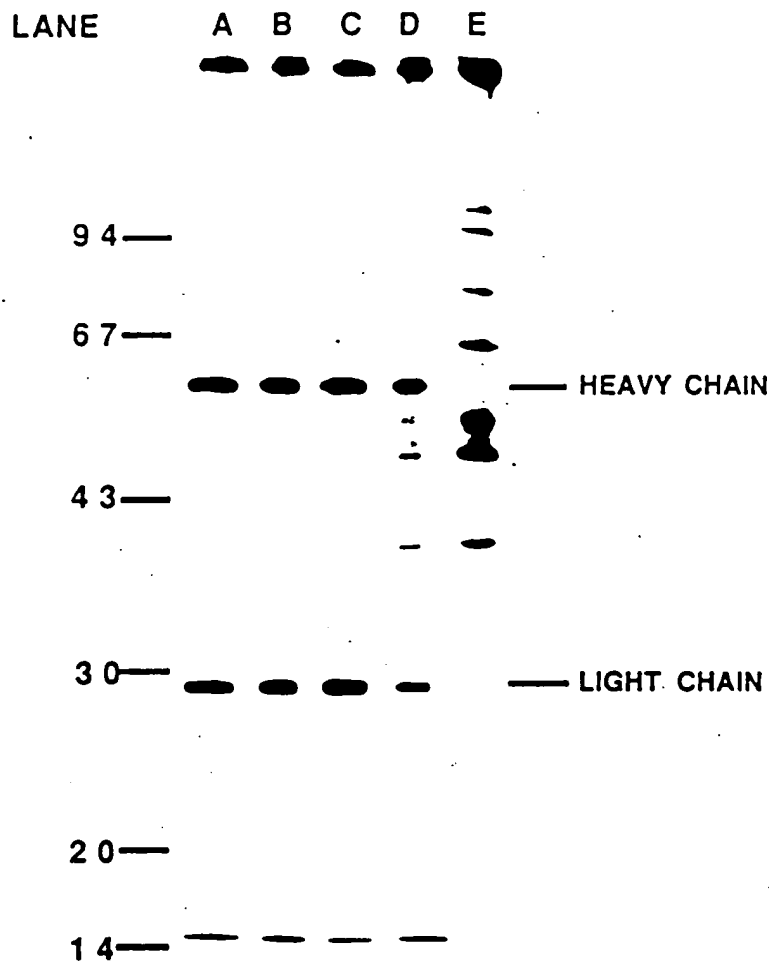
30
35

Fig 26. Effect on antibody expression of the presence of a "Kozak consensus sequence" immediately preceding the gH gene.

Reducing SDS-PAGE of ^{35}S labelled antibody purified from a COS cell transient expression experiment by Protein A-Sepharose precipitation.

KEY:

- A. - gH331 cL
- B. - gHK331 cL
- C. - gH341 cL
- D. - gHK341 cL
- E. - Mock transfection

A B C D E F G H I J K

Mol wt (kd)

94 —

67 —

43 —

30 —

20 —

14 —

— Heavy chain

— Light chain

Fig 27. Expression of gH chain genes with cL chain

Reducing SDS-PAGE of ^{35}S labelled antibody produced from COS cell transient expression experiment. Antibody was recovered from culture supernatant by binding to polyclonal anti-human F(ab')_2 and then by precipitation with Protein A-Sepharose.

KEY:

- A. cLcH OKT3
- B. gHK121 cL
- C. gHK131 cL
- D. gH141 cL
- E. gH321 cL
- F. gH331 cL
- G. gHK331 cL
- H. gH341 cL
- I. gHK341 cL
- J. gHK341B cL
- K. gHK341A cL

1. CDR GRAFTED LIGHT (gL) WITH MOUSE (mH) OR CHIMAERIC (cH) HEAVY CHAIN GENES

GENE COMBINATIONS	ANTIGEN BINDING	EXPRESSION
gL121 cH	-	+
KgL121A cH	+	+
gL221 cH	+/-	+
KgL221 cH	-	++
gL221A cH	+	+
KgL221A cH	+	++
gL221B cH	-	+
KgL221B cH	-	++
gL221C cH	+	+
KgL221C cH	+	++

2. CDR GRAFTED HEAVY (gH) WITH MOUSE (mL) OR CHIMAERIC (cL) LIGHT CHAIN GENES

GENE COMBINATIONS	ANTIGEN BINDING	EXPRESSION
KgH121 mL	not det.	-
KgH121 cL	not det.	-
KgH131 mL	not det.	-
KgH131 cL	not det.	-
gH141 mL	-	+/-
gH141 cL	-	+/-
gH321 cL	-	+
gH331 cL	-	+
KgH331 cL	-	+
gH341 mL	+	+
gH341 cL	+/-	+
KgH341 cL	+/-	+
KgH341A cL	+	+
KgH341B cL	+	+

3. FULLY CDR GRAFTED ANTIBODY

GENE COMBINATIONS	ANTIGEN BINDING	EXPRESSION
KgL221A KgH121	not det.	-
KgL221A KgH131	not det.	-
KgL221A gH141	not det.	-
KgL221A KgH331	not det.	-
KgL221A gH341	not det.	-
KgL221A KgH341	not det.	-
KgL221A KgH341A	+	+
KgL221A KgH341B	+	+

KEY	L	LIGHT CHAIN GENE (SEE TABLE 1 FOR NUMBER CODE)
	H	HEAVY CHAIN GENE
	m	MOUSE
	c	CHIMAERIC
	g	CDR GRAFTED
	K	PRESENCE OF KOZAK CONSENSUS SEQUENCE
	not det.	NOT DETERMINED (EXPRESSION LEVELS TOO LOW)

TABLE 2

A summary of the expression and antigen binding data for the CDR grafted genes constructed in this study

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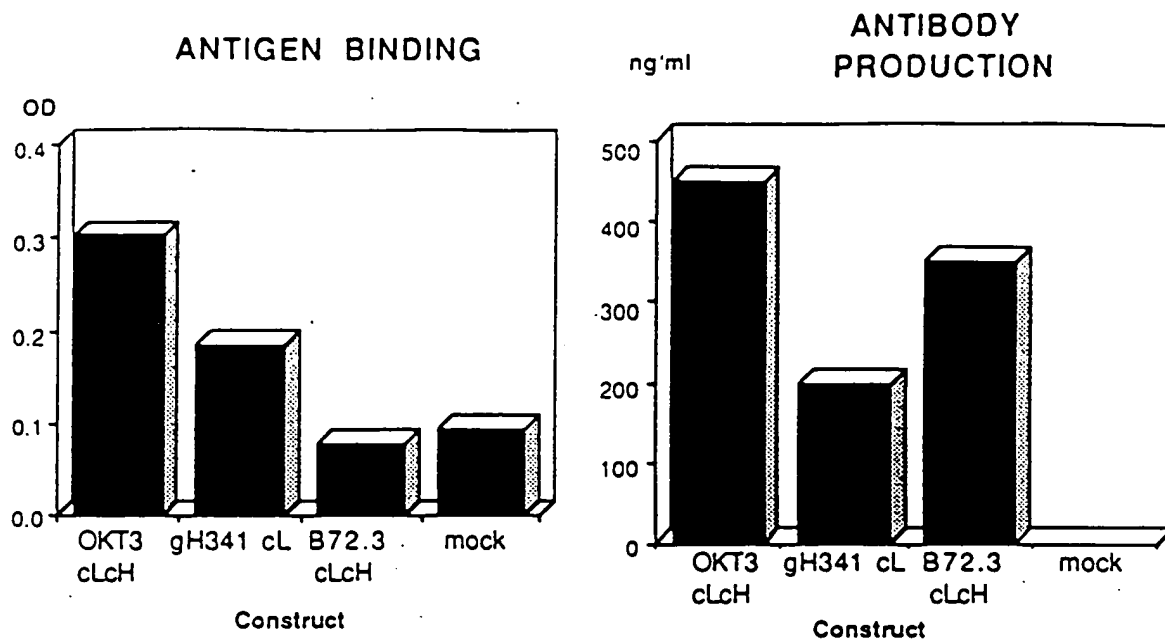


Fig 28 Antigen binding assay for gH341 cL gene combination.

Culture supernatants from COS cell transient expression experiments were tested for binding to Hut-78 cells (panel A) and for yield of assembled antibody (panel B). Chimaeric B72.3 was included as a negative control.

Note. Poor expression of gH341 cL gene combination.

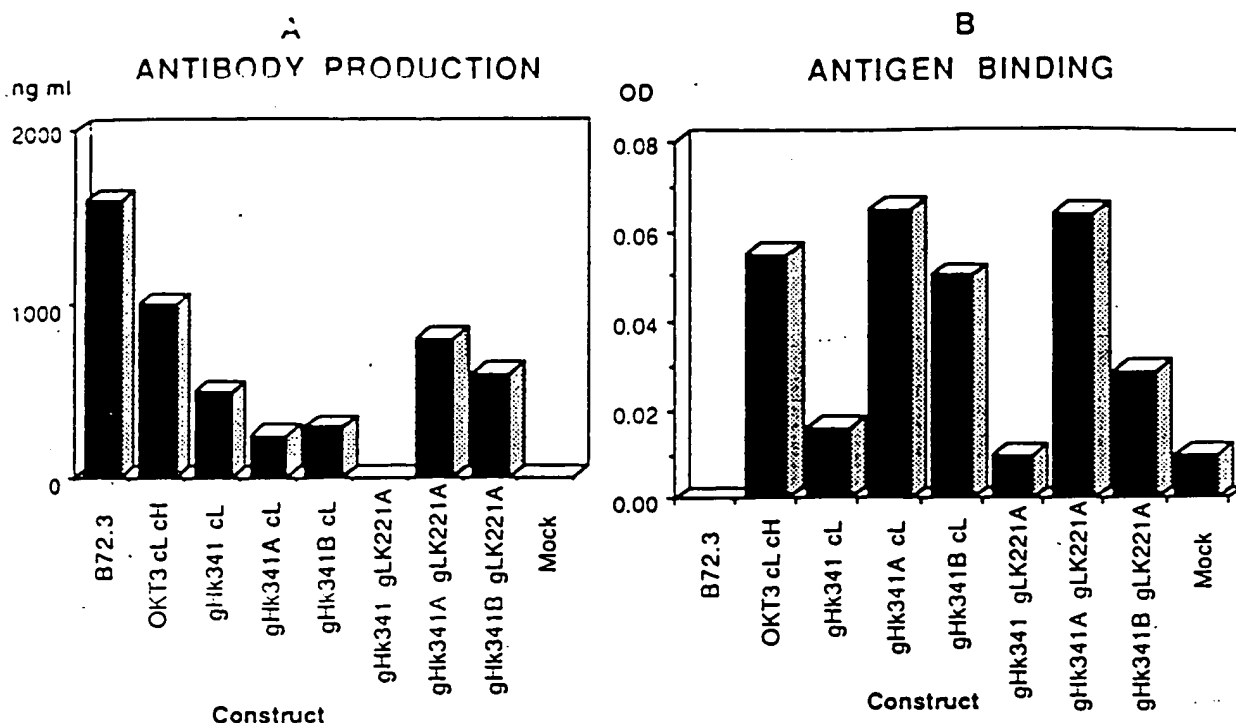


Fig 29 Antigen Binding assay for grafted OKT3 combinations

Culture supernatant from COS cell transient expression experiments were tested for yield of assembled antibody (Panel A) and for binding to Hut 78 cells (Panel B). Chimaeric B72.3 was included as a negative control. Panels show level of antibody produced and resultant antigen binding for various combinations of heavy and light chain genes cotransfected into COS cells.

NB: In panel B binding data has been normalised so that the level of binding B72.3 is set to zero.

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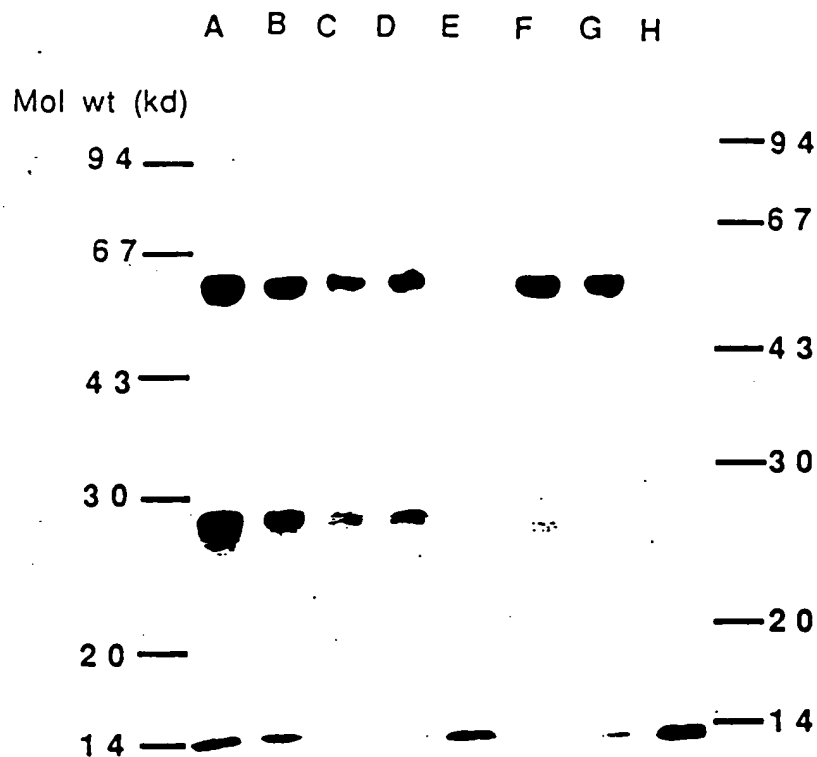


Fig 29c. gH341 series cotransfected with cL and gLK221A

Key:

- A. cL cH OKT3
- B. gHK341 cL
- C. gHK341A cL
- D. gHK341B cL
- E. gHK341 gLK221A
- F. gHK341A gLK221A
- G. gHK341B gLK221A
- H. Mock transfection